1996). Studies by Asal et al. (1988), Partanen et al. (1991), McCredie and Stewart (1993), Auperin et al. (1994), Chow et al. (1994), Mellemgaard et al. (1994), Mandel et al. (1995), Vamvakas et al. (1998), and Parent et al. (2000) controlled for at least smoking and body mass index. Because it is unlikely that exposure to trichloroethylene is associated with these factors, the committee judges that they do not significantly affect the estimates of risk. However, it might be useful in the risk assessment to distinguish and compare estimates of risk between studies in which confounding was and was not accounted for.

One possible source of bias that affects all case-control studies to some degree is the nonrepresentativeness of the study population to the target population. This can occur through sampling variation (essentially a random error, which is reflected in the estimates of variance and confidence intervals) and also through systematic effects in which subjects are recruited in a nonrandom way that might depend on exposure status (selection bias). In most case-control studies, the sampling fraction for cases is close to 100%, so that any selection biases would be manifest only if selection probabilities in the control series varied with exposure. However, low response rates can affect the validity of the findings, especially if they are also associated with exposure. The committee did not inspect the case-control studies for possible selection biases, but this is a necessary step in the risk assessment. In addition, the committee did not evaluate every study with regard to response rates, except noting low response rates in some studies (e.g., Partanen et al. 1991; McCredie and Stewart 1993) and those studies in which response rates were not stated (Asal et al. 1988; Harrington et al. 1989; Mandel et al. 1995).

Exposure Assessment

Table 3-10 presents the essential characteristics of exposure for selected case-control studies. The committee has general concerns about studies that use self-reports of exposures to occupational agents, especially in large urban centers where the prevalence of any one exposure would be low (Sharpe et al. 1989; Mandel et al. 1995; Schlehofer et al. 1995). In contrast to personal characteristics, such as age and race, for which subjects' reports are the gold standard, most individuals do not know what agents they used, especially if the agent occurred as part of a mixture or if a wide range of materials were used. On the other hand, most workers probably would know if they used a degreasing solvent.

The committee recognizes that there are special situations in which subjects are aware of their exposure circumstances. Questions about observable, objective facts tend to be answered accurately, such as "was there visible smoke in the air?" In workplaces where one or two materials are widely used, workers often know their common names, such as TCE, "Perc" (tetrachloroethylene), or Stoddard solvent, because there are limited choices and it makes a difference in the workplace which one is used. It is likely that workers enrolled in the study by Vamvakas et al. (1998) knew what solvent they were using; trichloroethylene was the solvent of choice for degreasing because it evaporates rapidly.

As with the cohort studies, an important factor that affects causal inference is how exposure is measured and assigned (validity, reliability) and whether exposure-response relationships are measurable. In studies in which analyses were conducted only by job title (Asal et al. 1988; Jensen et al. 1988; McCredie and Stewart 1993; Aupérin et al. 1994; Mellemgaard et al. 1994; Mandel et al. 1995), there would have been substantial misclassification that would have attenuated rate ratios. In addition, in some studies exposure was attributed by expert

TABLE 3-10 Characteristics of the Assessment of Exposure to Trichloroethylene in Selected Case-Control Studies

Study	Qualitative Assessment	Information on Settings	Duration	Exposure Quantification	Exposure Extrapolation	Dose Metrics
Vamvakas et al. 1998 (follow-up to Henschler study) (pending legal cases for workers' compensation—possible bias?)	Small companies making metal parts in Arnsberg area (no cases from Henschler case group); TCE used for cleaning; high prevalence of companies in area; some use of Perc; qualitative assessment by occupational hygienists and physicians, interviews.	Detailed info on TCE use, area descriptors—hot dip baths, small work areas, jobs, tasks—extensive cleaning; no controls.	Questionnaire, interviews, median 33 yr observation, sufficient latency.	None (frequency and severity of neurological symptom reports used to estimate high exposures; >200 ppm likely).	None in paper (Cherrie et al. [2001] extrapolated based on details in paper: peaks 400-600 ppm, long-term about 100 ppm).	Combination of symptom severity and frequency and duration of exposures ranked +, low; ++, medium; +++, high.
Bruning et al. 2003 (follow-up to Vamvakas study)	Small companies making metal parts in Arnsberg area (no cases from Vamvakas case group); TCE used for cleaning; high prevalence of companies in area; some use of Perc; qualitative assessment by occupational hygienists.	Detailed info on TCE use, area descriptors—hot dip baths, and small work areas, jobs, tasks—extensive cleaning; no controls.	Questionnaire, interview job histories and next of kin; median 33 yr observation, sufficient latency.	None (self-reported exposures, and two JEMs, CAREX, and British; >200 ppm likely for symptom reports).	None in paper (Cherrie et al. [2001] extrapolated based on details in paper: peaks 400-600 ppm, long-term about 100 ppm).	Job and industry groups with exposures from JEM; self-reported exposure and symptom frequency.
Brauch et al 1999; 2004 (molecular study of von Hippel-Lindau gene mutations in subjects highly exposed to TCE)	TCE used up to 1970s. Questionnaire by personal interview; secondary questions if TCE reported; employer records, hygienists from insurance companies.	Same plants as in Vamvakas study. Detailed info on TCE use, area descriptors—hot dip baths, small work areas, jobs, tasks—extensive cleaning; no controls.	Questionnaire, interview job histories and next of kin.	Scheme developed for cases by Vamvakas et al. (1998).	None in paper (Cherrie et al. [2001] extrapolated based on details in paper: peaks 400-600 ppm, long-term about 100 ppm).	Combination of symptom severity and frequency of exposures; ranked +, low; ++, medium; +++, high.

TABLE 3-10 *Continued*

Study	Qualitative Assessment	Information on Settings	Duration	Exposure Quantification	Exposure Extrapolation	Dose Metrics
Pesch et al. 2000a,b (incidence study)	Broad community study of five regions in Germany (included Arnsberg area).	Limited data on exposure setting; self-assessed exposure.	Questionnaire, interview job histories, agent use, and tasks.	Broad German and British JEMs, and local JEM based on job titles and tasks using self-reported information.	Expert judgment for who was exposed. No quantitative estimates.	Ever exposed; agent index based on duration, intensity, and probability of exposure.

Abbreviations: JEM, job exposure matrix; Perc, tetrachloroethylene; TCE, trichloroethylene.

assessments or through the use of job-exposure matrices, but the assessments were restricted to exposures to any solvents without identifying which were used (Harrington et al. 1989; Partanen et al. 1991; Dosemeci et al. 1999). In other studies, subjects reported whether they were exposed to specific agents (Sharpe et al. 1989; Mandel et al. 1995; Schlehofer et al. 1995; Vamvakas et al. 1998; Pesch et al. 2000a), and in some of those studies (e.g., Sharpe et al. 1989) rate ratios could have been overestimated because cases overreported exposure. In other studies, trichloroethylene was assessed specifically (Dosemeci et al. 1999; Parent et al. 2000; Pesch et al. 2000a; Brüning et al. 2003) and those studies may be more informative. In any risk assessment, the validity and reliability of the assessments of exposure have to be investigated closely.

Two small case-control studies conducted in the Arnsberg area of Germany found excess risks for exposure to trichloroethylene (Vamvakas et al. 1998; Brüning et al. 2003). The Vamvakas et al. (1998) study compared 58 renal cell cancer cases diagnosed between 1987 and 1992 with controls to provide an independent assessment of the Henschler et al. (1995) study. None of the cases in this study was included in the Henschler study. The odds ratios (OR) increased dramatically (OR = 1, 6.61, 11.92, and 11.42) with increasing exposures (categories of "no exposure," "+," "++," and "+++", respectively, based on exposure types, duration, and extent of prenarcotic symptoms). As summarized in Table 3-11, there were large differences in the severity of symptoms and duration of exposure by the amount of exposure. There were also large differences in the source of exposure, with the use of hot dip tanks (major sources of trichloroethylene vapors) predominating in the highest category and largely rag-and-bucket cleaning (limited local sources of trichloroethylene vapors) in the lowest category, which are consistent with large differences in the intensity of exposure. Thus, there is a high degree of consistency between symptom severity and reports of exposures.

TABLE 3-11 Prenarcotic Symptoms and Exposure Duration and Intensity Associated with Rated Exposure Levels in the Vamvakas et al. (1999) Study

	Rated Exposure Level		
Descriptor	+	++	+++
Prenarcotic symptoms (number, # category[a])	1, #1 3, #0	4, #3 9, #2	8, #3 2, #2
Frequency (number, daily or times per week)	1, daily 3, none	4, daily 4, 2 times per wk 5, 1 time per wk	5, daily 1, 3 times per wk 4, 2 times per wk
Exposure types	— — 3 rag and bucket 1 polishing	2 hot clean, 9 cold dip tanks, 1 rag and bucket, 1 dry clean	7 hot dip tanks; 1 welding on tanks with residues 2 cold dip tanks
Total duration (range)	Mean, 1,850 hr (1,100-2,500 hr)	Mean 4,141 hr (650-9,800 hr)	Mean, 28,800 hr (2,300-78,000 hr)

[a]Symptom grades were as follows: #0, none; #1, light symptoms (light dizziness, modest headaches); #2, moderate symptoms (light daze, clear dizziness, headaches); #3, severe symptoms (daze vertigo, severe headaches, and nausea, which did not permit the subject to remain exposed).

Another case-control study by Brüning et al. (2003) extended the period of observation from 1992 to 2000 and used the same rating scheme for exposure to trichloroethylene but used an independent set of cases and controls drawn from a wider geographic area. The validity of the data gathered by questions about neurological symptoms, which were asked of subjects in the Vamvakas et al. study, is a concern because legal proceedings were in progress to compensate workers for damage to their health. Two important considerations suggest that the workers' reports are valid. First, the Vamvakas et al. scale did not rely only on neurological symptoms but also included an assessment of duration and exposure intensity associated with the particular activities. Second, there are a variety of sensory cues that taken together can distinguish low and high vapor concentrations in addition to neurological symptoms. For example, at low exposures the vapors are colorless, nonirritating, and not pungent, but with high concentrations (greater than several hundred ppm) there are a variety of sensory cues: the vapors are irritating, have a strong odor, and were found to produce reduced performance and central nervous system symptoms in human volunteers during experiments in the 1960s (Stopps and McLaughlin 1967; also see Chapter 6). Thus, the workers' neurological symptoms were associated with other less subtle sensory responses, and they were only one dimension of the exposure evaluation. More importantly, overreporting would introduce misclassification, which would reduce the association between the symptoms and exposure.

The study by Vamvakas et al. (1998) has also been criticized in the literature; the essential observations are as follows:

1. Omission of cases from the Henschler et al. (1995) study (noted Mandel and Kelsh 2001). The effect of including these cases would lead to even higher estimates of risk.
2. Including only cases who worked in small industries and not applying the same criteria to controls (noted by Mandel and Kelsh 2001), which might lead to an underestimate of exposure among the control subjects and, thus, the odds ratios may have been overestimated. The committee shares the concern about this type of selection bias.
3. Cases were selected from one hospital but controls were selected from other hospitals in the area (noted by Green and Lash 1999; Mandel and Kelsh 2001). The committee is sanguine with regard to the selection of controls from other hospitals as it accepts the argument that these hospitals specialized in the type of care that they provided.
4. Prevalent cases (1987-1992) and controls (residual noncases) were selected in 1993 and interviews were conducted in 1993 (noted by Green and Lash 1999). McLaughlin and Blot (1997) suggested that survival in this period of time was 50% to 60%. Thus, some cases might have died in the interim before they could be interviewed and would have been excluded. This could have led to an inaccurate estimate of the exposure distribution. On the other hand, the control subjects who were enrolled when the interviews were conducted might not represent the true exposure distribution of the target population through time. In particular, exposures among the controls could have been underestimated if exposure diminished with time and if the selection of controls did not fully represent the actual distribution in the past (e.g., through changes in the population (immigration or emigration). Although this is a conjecture and the effects are difficult to predict, a sound design would have attempted to minimize such distortions. Thus, the committee is concerned about the possibility of a selection bias in this study and about the quality of the data obtained from subjects diagnosed in the past, especially if self-reported exposures were partially the basis of the assessments of exposure to trichloroethylene.

5. Interviewers (physicians) were aware of subjects' case status and surrogate respondents were used for deceased cases (but not for controls) (noted by Mandel and Kelsh 2001). One might expect that exposure of case subjects could have been overestimated because physicians more aggressively sought symptoms and exposure reports, although this was not possible for the deceased cases. It is unclear what overall effect this would have on the findings, although an analysis excluding the deceased cases would be useful.

6. Although the concentrations of exposure are unknown, the committee's analysis of the data in the Vamvakas et al. (1998) study, presented in Table 3-12 (see Appendix D for more detailed analysis of the this study), makes it clear that the severity of symptoms and the severity and duration of exposures were all substantial and consistent for the cases, and the controls as a group had fewer symptoms and lower exposures. The committee disagrees with the conclusions of some critics (Green and Lash 1999; Cherrie et al. 2001; Mandel and Kelsh 2001) that it was unclear how exposure to trichloroethylene was assessed. Table 3-12, prepared from the data of Vamvakas et al. (1998), clearly shows that graded differences on several scales are consistent with the ratings. Thus, a clear ordinal scale is present. However, the precise magnitude of exposures associated with these ratings is difficult to assess. Cherrie et al. (2001) separately estimated the exposure intensities with a suitable engineering model, which estimated peak exposures in the range of 500 ppm and averages about 100 ppm. The committee agrees with this assessment (see Appendix D). These exposures were consistent with the symptom reports in laboratory studies (Stopps and McLaughlin 1967).

TABLE 3-12 Trichloroethylene Exposure Summary for the Arnsberg Area Studies

Study	Peak Exposures	Long-term Exposures	Notes
Henschler et al. 1995	>2,000 ppm, machine cleaning with neurological symptoms; about 100 ppm continuous cold cleaning.	100 ppm 100 ppm	Cherrie et al. (2001) estimates
Vamvakas et al. 1998	400-600 ppm hot cleaning with neurological symptoms.	100 ppm	Cherrie et al. (2001) estimates
Bruning et al. 2003	400-600 ppm hot cleaning, with neurological symptoms.	100 ppm	

7. The control subjects were younger than the cases, implying a different potential for exposure to trichloroethylene (noted by Green and Lash 1999); therefore, risks could have been overestimated. If amounts of exposure have decreased and workers entered the workforce at about the same age across calendar periods, this could lead to an underestimate of exposure among controls, thereby leading to overstated risk ratios. However, responding to that criticism, the authors noted that there were no changes in exposure before 1986, when the allowable exposure was regulated. Given that the small enterprises have the highest exposures (Raaschou-Nielsen et al. 2002) and are usually the last to respond to regulations because of their limited resources, large employers are the initial focus of regulator activity. Further, the comment that younger workers would have lower exposures is not generally true because apprentices usually do the least skilled, dirtiest jobs; in the United States and Europe, younger workers have the highest exposures.

8. The authors did not find the two main accepted risk factors for renal cancer (smoking and obesity) (Mandel and Kelsh 2001). The main risk factors appear to be weakly associated with renal cancer, so not identifying these associations could be due to chance, lack of statistical power, or possibly to homogeneity of the population.

The study by Brüning et al. (2003) was carried out in a broader region of southern Germany, which included the Arnsberg region, by the same team of investigators but covered the calendar period 1992-2000 and a different set of cases and controls. Again, prevalent cases of renal cell carcinoma were identified in 1992-2000 and interviews were conducted in 1999-2000. Controls were identified and interviewed in 1999-2000 and were recruited from noncancer patients having surgery and from a local department of geriatrics. The geriatric department was used to enroll controls for patients who were older. Exposure was assessed on the basis of occupational history and self-reports of exposure to trichloroethylene and tetrachloroethylene, reports of prenarcotic symptoms for peak exposures using the same scheme as that in the Vamvakas et al. study, and the job exposure matrix of Pannett and the CAREX system to infer exposures. The committee judges that exposure range was likely similar to that in the Vamvakas et al. study, although they were drawing cases from a wider base population, which had a lower prevalence of exposures. For "ever exposed" to trichloroethylene, the investigators observed an OR of 2.47 and almost a 6-fold increase in risk among subjects who had daily occurrences of narcotic symptoms.

Some criticisms of this study are similar to those of the Vamvakas et al. study: (1) use of prevalent cases and residual noncases; (2) questions about the specific secondary study base that was used (surgery, geriatric clinics) and how representative it was of the target population; (3) whether interviewers were blinded and whether any of the authors were interviewers; and (4) whether surrogate respondents were interviewed as controls for deceased cases. In addition, although subjects were matched, there were noticeable differences in age (median age of cases, 68 years; median age of controls, 66 years).

The study by Vamvakas et al. exhibited a very large estimated OR of 10.8. The committee has concerns that the true exposure distribution of the target population was underestimated in the enrolled control series (criticisms 2, 4, 5, and 7 described above). Given the very large estimates of risk, a sensitivity analysis is warranted if these data are to be used in a risk assessment. The follow-up case-control study of Brüning et al. showed an OR of 2.47 for the same type of self-reported exposure to trichloroethylene but a broader more heterogeneous base population; the OR for jobs involved in metal degreasing that had potential exposure to trichloroethylene and tetrachloroethylene was 5.57. As the committee evaluated that the assessment of exposure in this study was similar to that of Vamvakas et al., this lower odds ratio might indicate bias in the Vamvakas et al. study or statistical variation between studies because the Brüning et al. study included a broader base population than that of the Vamvakas et al. and the Henschler et al. (1995) studies, which could have entailed a greater extent of misclassification of exposures. Despite these issues, the committee was impressed that three studies of the Arnsberg region of Germany, with very high apparent exposures and different base populations, showed a significant elevation of risk.

If there is doubt about the validity of a study, then the risk assessment can be conducted by including and excluding that study and determining the sensitivity of the findings. With regard to the study by Henschler et al., which shows much higher risks than the others, sensitivity analyses are warranted and their need can be argued by analogy to the standard

practice in epidemiology of assessing the effects of outliers; in risk assessment, this would be equivalent to the assessment of heterogeneity, except that the issue of a biased variance in the Henschler et al. study needs to be addressed.

Genetic Mutations and Kidney Cancer

Bruning et al. (1997a) described a possible somatic mutation in the Von Hippel-Lindau (*VHL*) tumor suppressor gene in the etiology of renal cell carcinoma arising from exposure to trichloroethylene. Brauch and coworkers have reported additional studies (Brauch et al. 1999; 2004). Brauch et al. (1999) compared somatic mutations among 44 cases with documented exposure to trichloroethylene in metal-processing plants in the Arnsberg region of Germany with 107 cases who had no such occupational exposure. The results of the study are summarized in Table 3-13. In addition, mutations at nucleotide 454 were found in 7 of the 17 high-exposure subjects and six of the 24 medium-exposure subjects, but no mutations were found in the three low-exposure patients or in 107 unexposed subjects.

Few details were presented about how subjects were selected, although they may have been recruited from the living subjects of the Vamvakas et al. (1998) case group. Whether there was blinding of exposure status in the assessments of somatic mutations was not stated.

In a second study, Brauch et al. (2004) reanalyzed cases from the Vamvakas et al. (1998) study for mutations in the *VHL* somatic gene. Thirty-eight of the original 58 patients with renal cell carcinoma were analyzed, and the authors used the original Vamvakas et al. exposure classification. Of the 17 exposed cases 15 had mutations and among the 21 unexposed cases 2 were found to have mutations (OR = 71.3). Because it was unclear to the committee why only this subset of cases was analyzed, a simple sensitivity analysis was conducted in which it was assumed that all 20 cases who were excluded were exposed but did not have any mutations. This analysis, which assumes extreme selection bias, still led to an OR of 6.5. An advantage of this "case-only" analysis is that it does not require use of the control series (analogous to a case-only study in gene-environment interactions); it was unclear whether there was blinding of exposure status when the molecular analyses were conducted.

TABLE 3-13 Number of Exposed and Unexposed Patients with *VHL* Gene Mutations

	VHL Mutational Status			
Trichloroethylene Exposure[a]	None	1	≥2	Total
High, +++	2	4	11	17
Medium, ++	6	15	3	24
Low, +	3	0	0	3
No documented occupational exposure[b]	31	42	0	73

[a]At the beginning of the discussion of the Brauch et al. (1999) paper it is noted that "The present study relies on the standardization of TRI [trichloroethylene] exposure levels of the RCC patients (10) …," where reference 10 is the Vamvakas et al. (1998) study; many of the coauthors are the same for both papers. Also in the paper by Brauch et al. (2004), p. 303, Table 1, a footnote notes that exposure data came from Vamvakas et al. (1998) subjects, and the case numbers of both Brauch et al. (1999, 2004) studies overlap. This indicates that the cases and their exposure assignments were obtained from the Vamvakas et al. (1998) study.

[b]The controls were drawn from other parts of Germany (populations assumed to be without the high prevalence of exposure to trichloroethylene) and evaluated by the same interview and questionnaire protocols.

Source: Adapated from Brauch et al. 1999.

Role of Metabolism in Trichloroethylene-Induced Renal Tumors

Extensive studies of trichloroethylene metabolism, coupled to its potential mechanism of action in nephrocarcinogenicity, have been reported (reviewed by Bruning and Bolt 2000). Trichloroethylene induces renal toxicity and renal tumors in rats (Maltoni et al. 1988; NTP 1988, 1990; EPA 2001). The nephrocarcinogenic effects of trichloroethylene are more pronounced in male rats, compared with female rats and were absent in male and female mice.[2] Studies of trichloroethylene metabolism in rodents and humans support a role for bioactivation in the development of nephrotoxicity and nephrocarcinogenicity after exposure to trichloroethylene (Lash et al. 1995, 2001a,b, 2002, 2003; Lash 2004). Trichloroethylene is metabolized by two competing pathways: oxidation by CYP450 and conjugation with glutathione (discussed earlier in this chapter; see Figure 3-1). Glutathione conjugation of trichloroethylene results in formation of *S*-(dichlorovinyl)glutathione, which is metabolized by enzymes of the mercapturic acid pathway (γ-glutamyl transpeptidase, aminopeptidase) to *S*-(1,2-dichlorovinyl)-L-cysteine, which is then metabolized by cysteine conjugate β-lyases, leading to the formation of electrophilic chlorothioketenes and sulfoxides. Concentrations of trichloroethylene in renal cortical homogenates have been reported to be generally two- to three-fold higher than in liver homogenates, and both oxidative and glutathione conjugation products were found in the liver and kidneys (Lash et al. 2006). These results are consistent with in vitro studies showing metabolism by kidney tissue. Males had substantially higher urinary excretion of *S*-(1,2-dichlorovinyl)-L-cysteine, suggesting greater metabolism by the glutathione pathway. It should be noted that results were reported for only three animals per time point and interpretation of the data is complicated by anomalous dose-concentration time profiles for trichloroethylene and its metabolites. The nephrotoxicity and nephrocarcinogenicity of trichloroethylene have been linked to the formation of *S*-(1,2-dichlorovinyl)-L-cysteine derivatives.

S-(1,2-Dichlorovinyl)-L-cysteine and its mercapturic acid metabolite *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine have been identified in the urine of humans exposed to trichloroethylene, providing evidence for the glutathione-dependent bioactivation of trichloroethylene in humans. Metabolism of trichloroethylene via the mercapturic acid metabolic pathway is consistent with the fact that the male rat is a sensitive species, because reduced glutathione (GSH) conjugation, γ-glutamyl transpeptidase, and cysteine conjugate β-lyase activity are all significantly higher in male than in female rats (Lash et al. 2002). Moreover, pharmacokinetic analysis of human volunteers after exposure to trichloroethylene (50 or 100 ppm) revealed that blood *S*-(dichlorovinyl)glutathione concentrations were 3.4-fold higher in males than in females, whereas clearance half-time values for systemic clearance of *S*-(dichlorovinyl)glutathione were similar in both genders (Lash et al. 1999). In the liver, metabolism of trichloroethylene via the mercapturic acid metabolic pathway is quantitatively less than via the CYP450-dependent metabolic pathway. However, the glutathione-dependent pathway becomes more pronounced when the oxidative metabolism of trichloroethylene is saturated in the case of high-dose exposure. Cummings and Lash (2000) demonstrated that human kidney tissue forms GSH conjugates with a K_m (0.58 mM) in the range of K_m values for oxidative metabolism by rodent microsomes (0.38 mM for mice, 0.07 and 0.48 mM for rats;

[2]Trichloroethylene is described in the literature as being carcinogenic in males only. The magnitude of effect is smaller in females and didn't reach statistical significance in the individual studies. EPA (2001) did an analysis of modified data. The results across strains were pooled and animals that died before any tumors were observed were removed from the analysis. With these modifications, the tumor effect in females was significant.

Table C-2). They reported minimal or nondetectable P450-mediated trichloroethylene metabolism in human kidney tissue.

Genotoxicity

Trichloroethylene causes a significant increase in the incidence of renal tumors in rats when administered orally and a marginal incidence of renal tumors when administered via inhalation; on the basis of limited evidence for carcinogenicity in humans and sufficient evidence for carcinogenicity in experimental animals, the International Agency for Research on Cancer (IARC 1995) classified trichloroethylene as a probable carcinogen in humans (group 2A). Moore and Harrington-Brock (2000) reviewed the genotoxicity of trichloroethylene and its glutathione-derived metabolites, and Bruning and Bolt (2000) reviewed the results of genotoxicity tests and concluded that trichloroethylene is, at most, a weak genotoxicant but noted that *S*-(1,2-dichlorovinyl)glutathione and *S*-(1,2-dichlorovinyl)-L-cysteine have genotoxic effects including mutagenicity in the Ames test, unscheduled DNA synthesis, and formation of adducts in vitro with adenine, cytosine, and guanine. In the preliminary screening phase, the standard battery of genotoxicity tests might be unable to identify tissue-specific carcinogens, if the test system lacks the enzymes needed to form the toxic metabolite, and certainly does not provide any information on the possible species specificity of the test compound (Brambilla and Martelli 2004; Moore and Harrington-Brock 2000). Recently, Robbiano et al. (2004) applied both in vitro and in vivo assays to measure genotoxicity to kidneys of rodents and repeated the assays in primary cultures of human kidney cells. Six chemicals known to induce kidney tumors in rats, including trichloroethylene, were examined for their ability to induce DNA fragmentation and the formation of micronuclei in primary cultures of rat and human kidney cells and in kidneys of intact rats. Each chemical was tested at three to six concentrations (four 2-fold dilutions for trichloroethylene); the highest concentration tested produced a less than 30% reduction in survival. Significant dose-dependent increases in the frequency of DNA single-strand breaks and alkali-labile sites (as measured by the Comet assay) and in micronuclei frequency were obtained in primary kidney cells from male rats and from humans of both genders, with subtoxic concentrations of trichloroethylene. Among the six test compounds (benzofuran, bromodichloromethane, captafol, nitrobenzene, ochratoxin A, and trichloroethylene), trichloroethylene and bromodichloromethane exhibited the lowest DNA-damaging and micronuclei-inducing potencies (with ochratoxin A exhibiting the highest) in rats and humans. In agreement with these findings, statistically significant increases in the average frequency of both DNA breaks and micronucleated cells were observed in the kidneys of rats given a single oral dose (half the lethal dose to 50% of rats) of the six test compounds. For all these effects, the magnitude of the response was among the greatest for trichloroethylene. The results of this study also showed that the six rat kidney carcinogens produced genotoxic effects in primary cultures of human kidney cells that were quantitatively and qualitatively similar to those observed in primary cultures of rat kidney cells. Taken together, these findings provide evidence that trichloroethylene is genotoxic in short-term genotoxicity assays in kidney cells isolated from rats and human donors.

However, the authors noted limitations in the experimental design that limit interpretation and the significance of the above studies (Robbiano et al. 2004). These limitations include (1) examination of trichloroethylene on cells from only three donors, (2) considerable variation in

the frequency of DNA lesions induced in the cells, and (3) the possibility that kidney cells derived from kidney cancer patients could be more sensitive to DNA-damaging activity due to a more marked expression of enzymes involved in the metabolic activation of kidney procarcinogens and suppression of DNA repair processes. Therefore, the results of the genotoxicity studies must be considered solely as indicating that trichloroethylene might be genotoxic to the human kidney; the authors suggest that the designation of "inadequate evidence for carcinogenicity to humans" might not be tenable in the absence of sufficiently powered and carefully controlled epidemiologic studies.

Mode of Action

Role of von Hippel-Lindau Tumor Suppressor Gene

Most of the studies thus far reviewed on the subject of the renal carcinogenicity of trichloroethylene rely on either epidemiologic approaches or on studies of trichloroethylene metabolism and toxicity. The development of DNA technology and the discovery of tumor suppressor genes opened up a new route of investigation on the potential carcinogenic effects of trichloroethylene. Mutation or inactivation of the *p53* tumor suppressor gene is a common genetic alteration in human cancers. However, the *p53* gene is not a target in human and rat renal cell carcinoma (Reiter et al. 1993; Nishiyama et al. 1995). Inactivation of the *VHL* tumor suppressor gene in humans is responsible for the hereditary *VHL* cancer syndrome, predisposing affected individuals to a variety of tumors in specific target organs. More than 80% of sporadic renal cell carcinoma, but not papillary renal cell carcinoma, is associated with inactivation of the *VHL* gene (Gnarra et al. 1994). The *VHL* gene is only infrequently involved in extrarenal neoplasms, despite the broad range of *VHL* mRNA expression (including brain, adrenal, prostate, and lung), suggesting that its function as a tumor suppressor gene is specific for kidney epithelial cells (Walker 1998). The protein product of the *VHL* gene appears to regulate cell cycle arrest (transition from G_1 to G_0) by stabilizing the cyclin-dependent kinase inhibitor p27 (Soucek et al. 1998). Although the *VHL* gene, which is commonly mutated in human renal cell carcinoma, does not appear to be involved in rat renal cell carcinoma (Walker et al. 1996), it shares a common downstream effector (p27 that controls cell cycle progression) with the *TSC2* gene, a genetic target of renal cell carcinoma development in the rat. Because *VHL* is not a target gene in rodent models of chemical-induced or spontaneous renal carcinogenesis, future animal studies should use models in which target genes share common downstream signaling pathways with *VHL*.

One paper has linked the *VHL* gene to chemical-induced carcinogenesis. Shiao et al. (1998) demonstrated *VHL* gene somatic mutations in *N*-nitrosodimethylamine-induced rat kidney cancers that were of the clear cell type. The clear cell phenotype is rare in rat kidney cancers, but it was the only the clear cell cancers that showed *VHL* somatic mutation. This provided an additional link between *VHL* inactivation and clear cell kidney cancer.

Brauch et al. (1999, 2004) analyzed renal cancer cell tissues for mutations of the *VHL* gene and reported increased occurrence of mutations in patients exposed to high concentrations of trichloroethylene. In the first study (Brauch et al. 1999), subjects were identified from an occupational trichloroethylene exposure registry. They found multiple mutations in 42% of the exposed patients who experienced any mutation and 57% showed loss of heterozygosity. A hot

spot mutation of cytosine to thymine at nucleotide 454 (C454T) was found in 39% of samples that had a *VHL* mutation and was not found in renal cell cancers from nonexposed patients or in lymphocyte DNA from either exposed or nonexposed cases or controls. As discussed earlier, little information was given on how subjects were selected and whether there was blinding of exposure status during the DNA analysis.

In the second study, Brauch et al. (2004) investigated 38 renal cell carcinoma patients from a previous German case-control study performed by Vamvakas et al. (1998). Brauch et al. compared different renal cell carcinoma patient groups (trichloroethylene-exposed versus non trichloroethylene-exposed patients). The Vamvakas et al. study had described differences in renal cell carcinoma risks between trichloroethylene-exposed (n = 17) and nonexposed patients (n = 21). Brauch et al. (2004) extended the analysis by comparing age at diagnosis and histopathologic parameters of tumors as well as somatic mutation characteristics in the *VHL* tumor suppressor gene. Renal cell carcinoma did not differ with respect to histopathologic characteristics in both patient groups. Comparing results from trichloroethylene-exposed and nonexposed patients revealed clear differences with respect to (1) frequency of somatic *VHL* mutations, (2) incidence of C454T transition, and (3) incidence of multiple mutations. The latter is an indication that the effect of trichloroethylene is not limited to clonal expansion of cells mutated by some other agent. The C454T hot spot mutation was exclusively detected in tumors from trichloroethylene-exposed patients, as were multiple mutations. Also the incidence of *VHL* mutations in the trichloroethylene-exposed group was at least 2-fold higher than in the nonexposed group.

Brauch et al. were not able to analyze all the samples from the Vamvakas study, in part because samples were no longer available. Using the data described by Brauch et al. (2004) (*VHL* mutation found in 15 exposed and 2 nonexposed individuals, and *VHL* mutation not found in 2 exposed and 19 unexposed individuals), an OR of 71.3 is calculated. The most extreme example would be to assume that all 20 cases who were excluded were exposed but did not have mutations in *VHL* (*VHL* mutations were found in 15 exposed and 2 unexposed individuals and *VHL* was not found in 22 exposed and 18 unexposed individuals), which leads to an OR of 6.5, which remains significant.

Collectively, the data support the concept of a genotoxic effect of trichloroethylene leading to *VHL* gene damage and subsequent occurrence of renal cell carcinoma in highly exposed subjects. All the evidence, taken together, provides a consistent and plausible mechanism for a causal relationship and is strongly supportive of trichloroethylene being a human carcinogen after long-term exposure to high doses, such as occupational exposures described in both studies conducted in Germany (Henschler et al. 1995; Vamvakas et al. 1998).

The *VHL* gene is commonly altered in kidney tumors, especially those with the clear cell phenotypes. The alterations include loss of the entire or a large part of the gene (>90%) and small base changes (30% to 60%), including insertions, deletions, and point mutations (Shiao 2004). These changes can lead to reduction of protein expression, protein truncation, and incorrect amino acids incorporated into the protein (also called missense mutation). Consequently, wild-type constitutive functions of *VHL* are inactivated, with the subsequent potential to initiate and to promote tumor development. However, different mutations might have distinct tumorigenic potentials. Frequent and diverse *VHL* mutations in sporadic renal cell carcinoma provide a sizable mutation spectrum that has been used to correlate with environmental exposures. The rationale of using genetic signature as a marker of environmental exposure has been strengthened by in vitro and in vivo studies. Correlating of specific mutations

within the *VHL* gene with certain environmental exposures could lend support to the potential mutagenicity of an agent. Identification of DNA damage unique to exposure is necessary to provide strong evidence for the mutagenic potential of an environmental agent. Many types of DNA damage have been shown to induce unique signatures of gene mutations (see Table 3-14).

A worldwide mutation database compiling *VHL* mutations in sporadic renal cell carcinoma showed that missense mutations compose about 29% of all mutations; a large majority of base changes (71%) are nonmissense, including insertions, deletions, and frameshift alterations (see Table 3-15). When bases were determined, G:C to A:T, A:T to G:C, and A:T to C:G composed 48% of the changes. Similar mutation spectra have been obtained from cells and animals treated with alkylating agents, such as nitrosamines found in tobacco smoke and potent

TABLE 3-14 Mutation Spectra Indicative of Environmental Exposures and DNA Damage

Base Change	Possible Causes
Transition	
G:C to A:T	Deamination of 5-methyl-C or C; alkylation of G at O^6 position
A:T to G:C	Deamination of A; alkylation of T at O^2 or O^4 position
Transversion	
G:C to T:A	Mispairing of A with 8-OH-G or with apurinic G
A:T to T:A	Mispairing of A with apurinic A site
A:T to C:G	Misincorporation of 8-OH-G; error-prone repair of O^2- or O^4-alkyl T
G:C to C:G	Mispairing of G with oxidatively damaged G

Source: Shiao 2004. Reprinted with permission; copyright 2004, National Cancer Institute at Frederick.

TABLE 3-15 *VHL* Mutations in Sporadic Renal Cell Carcinomas

	Bruning et al. 1997a	Brauch et al. 1999		Brauch et al. 2004		UMD[a]
	Trichloroethylene Exposure					
	Yes	Yes	No	Yes	No	Unknown
Number of patients	23	44	73	17	21	
Patients with mutations	23 (100%)	33 (75%)	42 (58%)	14 (82%)	2 (10%)	
Number of mutation	23[b]	50	42	24	2	222
Missense	1	27 (54%)	NA	17 (71%)	2 (100%)	64 (29%)
Nonmissense	3	23 (46%)	NA	7 (29%)	0 (0%)	158 (71%)
G:C to A:T	1	21 (78%)	NA	12 (71%)	1 (50%)	21 (25%)
C to T at 454		(13)	(0/107)	(9)	(0)	(0)
G:C to T:A		0	NA	0	0	19 (22%)
G:C to C:G		5 (19%)	NA	4 (24%)	0	16 (19%)
A:T to T:A		1 (4%)	NA	1 (6%)	0	9 (11%)
A:T to G:C		0	NA	0	1(50%)	14 (16%)
A:T to C:G		0	NA	0	0	6 (7%)

Abbreviation: NA, not applicable.
[a]Universal Mutation Database (Beroud et al. 2000).
[b]By single strand conformation polymorphism (4 sequences confirmed).

human and animal renal carcinogens. The involvement of alkylating agents in the causation of renal cell carcinoma is further supported by the isolation of O^6-methylguanine and other alkylated DNA-damaged bases. However, mutation spectra after exposure to trichloroethylene or analog compounds, in cells and animals, have not been consistent. Nonetheless, increases in GC to AT and GC to TA mutations have been observed in bacteria. Muller et al. (1998) identified cytosine adducts from haloketene and halothioketene products of trichloroethylene; these are structurally similar to hydroxylamine cytosine adducts that result in C to T mutations (Budowsky 1976). Increases in *VHL* missense mutations, predominant in G:C to A:T base changes, and a hot spot of mutation at nucleotide 454, correlated with trichloroethylene exposure (Brauch et al. 1999). The three reports of trichloroethylene exposure from the same group suggest that trichloroethylene increases *VHL* mutations and generates a unique genetic signature of trichloroethylene exposure, which leads to the development of renal cell carcinoma. Although the findings linking trichloroethylene to renal cancer are of great consequence and relevance, further confirmation of mutagenicity and carcinogenicity at the molecular level is required to confirm the initial observations. As discussed earlier, consensus for the mutagenicity of trichloroethylene in mammalian cells remains to be established. If the mutation spectra in bacteria are considered, one would expect to see increases of both G:C to A:T and G:C to T:A mutations in trichloroethylene-exposed humans. However, a disproportionate number of G:C to A:T *VHL* mutations were reported (Brauch et al. 1999). Because alkylating agents, present in patients exposed to tobacco smoke, diuretic treatment for hypertension, and long-term dialysis for end-stage renal failure, also induce the same G:C to A:T base changes, analysis of prior trichloroethylene studies need to adjust for these risk factors. The temporal relationship between various mutations in the *VHL* gene and renal tumor progression needs to be examined more critically to unequivocally evaluate the cause-and-effect relationships. The mutagenicity of trichloroethylene should also be validated in additional cohorts. Further, the tumorigenic potentials of various *VHL* mutations need to be integrated, because mutated bases need not always be carcinogenic.

It remains debatable whether alterations in *VHL* alone are sufficient to trigger tumorigenic processes in the kidney, especially since experiments failed to detect any tumors in *VHL* knockout mice (Gnarra et al. 1997; Haase et al. 2001). Studies attempting to link the *VHL* gene to kidney tumor development are continuing in a variety of experimental models (Shiao et al. 1997, 1998; Walker 1998). However, there does not appear to be an experimental animal model with which to investigate the effects of trichloroethylene-induced mutations in the *VHL* gene and kidney tumor development.

Role of Nephrotoxicity in Trichloroethylene Renal Cancer

In animal studies, renal cancer occurs at high doses and is preceded by nephrotoxicity affecting the proximal tubule (NTP 1988, 1990). This has led to the proposal that nephrotoxicity is a prerequisite for the development of renal tumors and that exposures below nephrotoxic concentrations pose no risk of cancer. That is, there is a threshold exposure below which nephrotoxicity, and therefore renal cancer, will not occur (Bruning and Bolt 2000; Harth et al. 2005). In this scenario, nephrotoxicity, and subsequent cell division repairing that damage, functions as a promoter, allowing the expression of mutations (either spontaneous or induced by exposure to other agents, such as smoking and diuretics) within the renal cortex. Alternatively,

trichloroethylene is a complete carcinogen, with nephrotoxicity as the promoter for cells initiated by a trichloroethylene metabolite. There is evidence that trichloroethylene is genotoxic to human cells (Robbiano et al. 2004).

Nephrotoxicity is almost certainly secondary to formation of a toxic metabolite, and species differences in the extent of formation of that toxic metabolite could render humans less likely to develop nephrotoxicity and therefore cancer. The CYP2E1 and -3A5 isoforms that metabolize trichloroethylene have polymorphisms within national populations, resulting in considerable interindividual differences of enzyme expression. On a practical level, the population diversity in bioactivation and detoxification abilities could effectively obscure any threshold.

Investigations of nephrotoxicity in human populations have been pursued and the results show that highly exposed workers experience a tubular type of proteinuria, evidence of damage to the proximal tubule (Bruning et al. 1999a,b; Bolt et al. 2004). What is not clear is the magnitude of exposure needed to produce kidney damage. The fact that proteinuria was found in workers exposed to trichloroethylene concentrations that were not measured but were described as current occupational exposures (Green et al. 2004) is inconsistent with nephrotoxicity occurring only at high exposures that are not relevant to current occupational exposures.

FINDINGS

Although the committee was not charged with performing a risk assessment, it became clear from the epidemiologic evidence that there were sufficient data to make a recommendation about whether the findings of the mortality and incidence studies provided support for or against the hypothesis that exposure to trichloroethylene was associated with the induction of kidney cancer. There is strong evidence that exposure to high doses of trichloroethylene is associated with increased rates of kidney cancer. In particular, support for this conclusion derives from findings of increased risks in a cohort study (Henschler et al. 1995) and in case-control studies from the Arnsburg region of Germany (Vamvakas et al. 1998; Pesch et al. 2000a; Brüning et al. 2003). The committee notes that, as the designs of these case-control studies improved with time, increased risks were still observed. In addition, the finding of a mutation in the *VHL* somatic gene adds strength to these observations, although it would be useful if this finding were replicated in other settings. Of considerable interest was the finding of an increased risk among workers of a cardboard manufacturing plant in the United States (Sinks et al. 1992), who might have had exposures comparable to that in the study by Henschler et al. (1995). Other studies with appropriate power to detect risks from relatively low exposures also showed increased risks, notably the studies by Dosemeci et al. (1999), Raaschou-Nielsen et al. (2003), and Zhao et al. (2005).

Supporting this conclusion is the concordance between studies on humans and experimental animals for the site of tumors and occurrence of toxicity. In bioassay studies, rats developed tubular toxicity before tumors developed. Nephrotoxicity preceding cancer also appears likely in humans, although nephrotoxicity assessments in human studies were not made until after the development of renal cancer and were based on only one parameter.

The committee reviewed studies on two modes of toxicity proposed to be linked to cancer—accumulation of $\alpha_{2\mu}$-globulin and PPAR agonism. The committee concluded the evidence demonstrates these modes do not occur for trichloroethylene-induced renal cancer. The

committee also concluded that trichloroethylene causes an increase in the urinary excretion of formate but notes the disparities between formate-production and toxicity contradicts the conclusion that accumulation of formate is a mode of action for trichloroethylene nephrotoxicity.

Studies with experimental animals and human tissues support the conclusion that trichloroethylene, via one or more of its metabolites, is genotoxic. In animal studies, trichloroethylene appears to be a weak genotoxicant. The studies with human tissues used a small number of samples and, therefore, the committee notes this weakens the weight of evidence.

In the kidney, trichloroethylene can act as a complete carcinogen (at the stages of both tumor initiation and tumor promotion and progression) in a dose-dependent manner. Different types of kidney cancer can be triggered by different genes. After the discovery of the *VHL* tumor suppressor gene, it became recognized that homozygous inactivation of the *VHL* gene was linked to the occurrence of renal clear-cell carcinoma, the renal carcinoma preferentially induced by trichloroethylene. In exposed subjects, the genotoxic effect of trichloroethylene likely results from bioactivation pathways leading to renal *VHL* gene damage and renal cell carcinomas. The findings of experimental, mechanistic, and epidemiologic studies lead to the conclusion that trichloroethylene can be considered a potential human carcinogen.

RESEARCH RECOMMENDATIONS

- Because sulfoxide metabolites are more potent nephrotoxicants than their parent *S*-conjugates, more research is needed on the extent of formation of *S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine sulfoxides by human tissues (liver and kidney), the extent to which these reactions occur in vivo, the enzymes involved, and their interindividual variability, including the role of genetic polymorphisms. The toxicologic significance of trichloroethylene or *S*-(1,2-dichlorovinyl)-L-cysteine *S*-conjugate sulfoxidation products also should be evaluated.
- High frequencies of missense mutations in the *VHL* gene do not constitute a cause of renal cell carcinoma; the tumorigenic potential of missense mutations in the *VHL* gene should be determined. The potential of specific missense mutations in the *VHL* gene contributing to tumor initiation and progression should be determined.
- Although correlation of *VHL* mutations to trichloroethylene exposure and renal cell cancer are persuasive, the findings need to be validated in other populations and geographic areas. Because many risk factors for renal cell carcinoma generate mutation spectra similar to that of trichloroethylene, coexposure to trichloroethylene with other risk factors needs to be seriously considered and accounted for in future epidemiologic studies.
- Mechanistic studies should include field studies of populations exposed to trichloroethylene to assess the range of metabolic pathways used and relative amounts of metabolites from each pathway as a function of exposure intensity and enzymatic genotypes. This information will greatly help in the interpretation and extrapolation of information from rodents to humans.
- Additional studies of nephrotoxicity in workers exposed occupationally to trichloroethylene should be performed. It is important that actual exposures are measured and not estimated using biological markers that are subject to large interindividual differences.

- No analytic community studies were included in the committee's assessment of kidney cancer. Given the importance of contamination of water supplies by trichloroethylene, it is important that sufficiently robust studies (with sufficient statistical power and exposure assessments) be conducted in the general population where such exposures might be occurring.
- Any follow-up epidemiologic study must have a wide range of exposures, preferably to the range of the Vamvakas and Henschler studies to provide an anchor in that range where effects were seen. There may be opportunities for studies of populations in developing countries in Asia and Eastern Europe, where high exposures to trichloroethylene may not have been controlled. Strong, quantitative exposure assessments will be critical for these studies to be useful for resolving the remaining dose-response issues.

4

Liver Toxicity and Cancer

This chapter reviews information on the effects of trichloroethylene and its principal metabolites (trichloroacetic acid, dichloroacetic acid, and chloral hydrate) on the liver, particularly information generated since the U.S. Environmental Protection Agency (EPA) released its draft health risk assessment (EPA 2001b). Trichloroethylene metabolism occurs primarily in the liver and is critical to understanding its toxicity and carcinogenicity. Background information on trichloroethylene metabolism is provided in Appendix C. In this chapter, hepatotoxicity and liver cancer are discussed separately, although they are not necessarily independent end points. A review of current knowledge on the proposed modes of action for trichloroethylene-induced liver cancer (peroxisome proliferator-activated receptor agonism, genotoxicity and mutagenicity) and their relevance to humans is provided.

HEPATOTOXICITY

Animal Studies

It is well documented that trichloroethylene produces hepatotoxicity in experimental animals and humans (ATSDR 1997a; EPA 2001b). Table 4-1 provides the details of some recent studies, and selected findings are discussed below.

Rodents exposed to high doses of trichloroethylene or some of its metabolites develop hepatocellular necrosis. Different studies have localized the injury to midzonal, periportal, or centrilobular hepatocytes (Buben and O'Flaherty 1985; Soni et al. 1998, 1999; Lee et al. 2000). This lack of consistency in location of injury might reflect the routes of administration, doses, strain, or species of rodents used in the different studies. For example, Soni et al. (1999) conducted dose-response studies with trichloroethylene (250-2,500 mg/kg) to investigate the time course of liver injury and compensatory hepatocyte regeneration. Hepatocellular necrosis was evident after 24 hours at all doses. Injury was detected in midzonal areas of the liver lobule with no evidence of necrosis in hepatocytes adjacent to the central vein (centrilobular hepatocytes). This study also showed that the dose of trichloroethylene can influence the location of injury. At a trichloroethylene dose of 2,500 mg/kg, centrilobular injury was clearly

TABLE 4-1 Hepatotoxicity of Trichloroethylene and Metabolites in Animal Studies

Species (Sex)	Doses/Concentrations	Duration of Exposure	Route/Vehicle	Features of Hepatotoxicity	Reference
Swiss-Cox mice (males)	0-3,200 mg/kg/day, TCE	6 wk	Gavage/corn oil	Increases in serum glutamic pyruvic transaminase evident only with the two highest doses; histologic findings: swollen hepatocytes and minimal evidence of necrosis.	Buben and O'Flaherty 1985
Sprague-Dawley rats (males)	250, 500,1,250, 2,500 mg/kg, TCE	Single dose	i.p./corn oil	Midzonal injury that spreads to centrilobular regions with the highest dose.	Soni et al. 1998, 1999
Sprague-Dawley rats (males)	16 and 64 mg/kg, TCE	Single dose	i.v. or via portal vein cannula/vegetable oil	Periportal necrosis with portal vein administration. No necrosis with i.v. administration. This is reflective of solvent-related injury of TCE to portal areas.	Lee et al. 2000
Autoimmune prone $MRL^{-/-}$ mice (female)	0, 0.1, 0.5, 2.5 mg/mL, TCE	4 and 32 wk	Drinking water	$CD4^+$ T-cell activation; mononuclear infiltration in portal regions consistent with autoimmune hepatitis; slight but significant increase of serum ALT.	Griffin et al. 2000a
Autoimmune prone $MRL^{-/-}$ mice (female)	0 or 2.5 mg/mL, TCE in presence of diallyl sulfide (CYP2E1 inhibitor)	4 wk	TCE in drinking water; diallyl sulfide via osmotic pump	Blockade of TCE protein adduct formation; reversal of $CD4^+$ T-cell-mediated autoimmunity by TCE.	Griffin et al. 2000b
Autoimmune prone $MRL^{-/-}$ mice (female)	0, 0.1, 0.9 mg/mL, TCA or chloral	4 wk	Drinking water	$CD4^+$ T-cell activation by these metabolites as shown for TCE.	Blossom et al. 2004
Wistar rats (male)	376 ppm TCE, 4 hr/day, 5 days/wk	8, 12, 24 wk	Inhalation	This dose (1/25 LC_{50}) resulted in hepatomegaly and fatty infiltration; fatty changes with marked necrosis were more evident at 12 and 24 wk. No elevation in serum liver transaminases or mortality was detected.	Kumar et al. 2001a

TABLE 4-1 *Continued*

Species (Sex)	Doses/Concentrations	Duration of Exposure	Route/Vehicle	Features of Hepatotoxicity	Reference
B6C3F$_1$ mice (female)	25, 50, and 100 mg/kg chloral hydrate, 5 days/wk	3, 6, 12 mo, 2 yr	Gavage/in water	No evidence of dose-dependent elevation in serum liver transaminases, only AST elevated at the 50-mg/kg dose.	NTP 2002b
Swiss-Webster mice (male)	15, 30, 75 mg/kg, DCVC	Single dose	i.p./in water	Transient elevation in liver transaminases at the highest dose; no histologic evidence of liver injury.	Vaidya et al. 2003a
B6CF$_1$ mice (male)	1 g/L chloral hydrate; 0.5 g/L, DCA	104 wk	Drinking water	Increased liver weight and hepatocellular necrosis with both metabolites.	Daniel et al. 1992
B6C3F$_1$ mice (male)	0.5 or 5g/L, DCA	0, 5, 15, 20, 30 days	Drinking water	Dose- and time-dependent liver enlargement; morphologic evidence of focal necrosis and apoptotic bodies.	Carter et al. 1995
B6C3F$_1$ mice (male and female)	1 or 2 g/L, DCA or TCA	52 wk	Drinking water	Enlarged livers, significant glycogen accumulation, focal areas of necrosis seen with DCA. No focal necrosis with TCA, modest hyperthrophy and glycogen accumulation.	Bull et al. 1990
B6C3F$_1$ mice (male and female)	1 or 2 g/L, DCA or TCA	52 wk	Drinking water	Hepatocytes from DCA-treated mice contained large amounts of glycogen evenly distributed throughout the liver; less glycogen accumulation with TCA treatment, which was more prominent in periportal regions.	Bull et al. 1990

TABLE 4-1 *Continued*

Species (Sex)	Doses/Concentrations	Duration of Exposure	Route/Vehicle	Features of Hepatotoxicity	Reference
B6C3F$_1$ mice (male)	0.1 to 3 g/L, DCA	1, 2 and 8 wk	Drinking water	Dose-dependent liver glycogen accumulation associated with decreased glycogen synthase activity. No effect in glycogen phosphorylase or glucose-6-phosphatase (enzymes involved glycogen metabolism).	Kato-Weinstein et al. 1998
B6C3F$_1$ mice (male)	0.1 to 2 g/L, DCA	2-10 wk	Drinking water	Significant reduction in serum insulin levels, insulin receptor expression, and protein kinase B. Increases in liver glycogen preceded these changes.	Lingohr et al. 2001
Fresh hepatocytes in culture from B6C3F$_1$ mice (male)	10-500 μM, DCA	16-hr incubation	N/A	Dose- and time-dependent glycogen accumulation. Presence or absence of insulin in culture media did not affect this DCA effect. However, glycogen accumulation is dependent on phosphatidylinositol 3-kinase activity.	Lingohr et al. 2002
B6C3F$_1$ mice (female)	3.2 g/L, DCA with or without L-methionine at 4 or 8 g/kg	8 and 44 wk	DCA in drinking water; L-methionine in diet	L-Methionine prevented liver DNA hypomethylation completely, while blocking only 25% of glycogen accumulation produced by DCA.	Pereira et al. 2004
Fisher 344 rats (male) and B6C3F$_1$ mice (female)	0, 1, 2 g/kg, dibromoacetic acid	2, 4, 7, 28 days	Drinking water	Dose- and time-dependent hypomethylation of liver DNA in both species. Significant increases in liver glycogen in both species, although longer exposure is required in rats for this effect.	Tao et al. 2004a

TABLE 4-1 *Continued*

Species (Sex)	Doses/Concentrations	Duration of Exposure	Route/Vehicle	Features of Hepatotoxicity	Reference
Sprague-Dawley rats (males)	0.01, 0.1, 1, 5, and 10 mmol/kg, TCE	Daily dosing for 3 days	i.p./corn oil	Increases in total and some individual serum bile acids (TC was most sensitive). No elevation in transaminases (except for ALT at the highest dose) or morphologic evidence of injury. Separate inhalation studies showed similar elevation in serum TC levels.	Wang and Stacey 1990
Fresh hepatocytes in culture from Sprague-Dawley rats (males)	Cells were dosed by vapor phase in 25-mL flasks; exposure: 0, 0.5, 1, 2, 4 μL per flask, TCE	20-min exposure	N/A	Dose-dependent inhibition of bile acid (TC) uptake into hepatocytes. Time-dependent inhibition of bile acid accumulation. No significant intracellular enzyme or potassium leakage. No changes in bile acid efflux from hepatocytes with TCE exposure.	Bai and Stacey 1993
Fresh hepatocytes in culture from Sprague-Dawley rats (males)	Cells were dosed by vapor phase in 25-mL flasks; exposure: 0, 2, 5, 10 μL per flask (concentration range: 230-1,000 μM), 1,1,1-trichloroethane	20-min exposure	N/A	No ALT, lactate dehydrogenase or potassium leakage at any dose level. Concentration-dependent inhibition of TC, ouabain, and 2-aminoisobutyric acid uptake. Decrease ATP levels and activity of ATP-dependent membrane ATPases. No overt morphologic changes in TCE-exposed hepatocytes.	Kukongviriyapan et al. 1990

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCA, dichloroacetic acid; DCVC, *S*-1,2-dichlorovinyl-L-cysteine; i.p., intraperitoneal; i.v., intravenous; LC_{50}, concentration lethal to 50% of test animals; N/A, not applicable; ppm, parts per million; TC, taurocholate; TCA, trichloroacetic acid; TCE, trichloroethylene.

evident after 24 hours. High doses of trichloroethylene administered into the portal vein caused periportal liver injury via a direct solvent action rather than a mechanism dependent on activation by the enzyme cytochrome P-450 (CYP-450) (Lee et al. 2000).

Reynolds and Moslen (1977) proposed that reactive intermediates of trichloroethylene generated by CYP-450 bind covalently to cellular components, resulting in cell necrosis. More recent evidence from mouse studies suggests that an autoimmune response might play a role in trichloroethylene-mediated liver disease (Griffin et al. 2000a). Administration of trichloroethylene at concentrations of 0 to 2.5 mg/mL in drinking water to autoimmune-prone $MRL^{+/+}$ mice for 34 wk resulted in an inflammatory response in the liver. Metabolic activation of trichloroethylene by CYP2E1 was demonstrated to be an obligatory step in the development of autoimmune hepatitis in the mice. The metabolites trichloroacetic acid and chloral hydrate also have the potential to induce autoimmunity in the same autoimmune-prone mice (Blossom et al. 2004).

Recent studies investigated the hepatotoxicity produced by trichloroethylene in rats exposed via inhalation at 376 parts per million for 8, 12, or 24 wk. Liver enlargement with necrotic cells and fatty infiltration was more prominent in rats in the 12- and 24-week treatment groups. The authors also detected elevated markers of lysosomal disruption. They recorded no mortality in any of the treatment groups (Kumar et al. 2001a).

Human Studies

Table 4-2 presents findings from human studies of hepatotoxicity. There is some evidence that occupational exposure to trichloroethylene results in several forms of non-cancer liver disease such as hepatic necrosis, fatty liver, and cirrhosis. It is well established that acute occupational exposure to trichloroethylene does not produce liver injury, whereas chronic exposure does. Case reports have linked occupational exposure to trichloroethylene with Stevens-Johnson syndrome (erythema multiforme major) of abrupt onset (Phoon et al. 1984). All these cases demonstrated liver involvement ranging from mild jaundice to fatal liver failure. Another case report documented that repeated exposure to trichloroethylene in the work setting resulted in chronic cirrhosis and portal hypertension (Thiele et al. 1982). The most recent report of trichloroethylene hepatotoxicity associated with occupational exposure comes from a watch manufacturing plant in Thailand, where two female workers developed generalized skin lesions, fever, and hepatitis. One case resulted in fatal hepatic damage 2 weeks after the onset of symptoms. Both workers cleaned watch metal straps by dipping them in containers that contained trichloroethylene (Pantucharoensri et al. 2004).

These case reports support data from animal studies indicating that an autoimmune response might be important in trichloroethylene-induced hepatitis. Genetic and environmental factors that influence xenobiotic metabolizing enzymes can favor the formation of trichloroethylene metabolites capable of triggering an immune response against the liver.

Contribution of Metabolites to Hepatotoxicity

Chloral hydrate, a metabolic intermediate of trichloroethylene, has been reported to contribute to the hepatotoxic potential of this solvent. In a 2-year National Toxicology Program

TABLE 4-2 Hepatotoxicity of Trichloroethylene and Metabolites in Human Studies

Subjects	Doses/Concentrations	Duration of Exposure	Route/Vehicle	Features of Hepatotoxicity	Reference
Five case reports (males and females)	50-912 mg/m^3, TCE	3-5 wk	Inhalation of vapors in workplace	Stevens-Johnson syndrome (erythema multiforme), jaundice, hepatomegaly, and hepatic encephalopathy. Other solvents besides TCE might be involved.	Phoon et al. 1984
Two case reports (females)	15-45 ppm, TCE	4-5 wk	Inhalation of vapors in workplace	Stevens-Johnson syndrome, generalized skin eruptions, and hepatitis with no jaundice (case 1). Fulminant hepatitis (case 2).	Pantucharoensri et al. 2004
Cross-sectional study (148 workers) and a 2-yr follow-up study (13 workers)	Low, moderate, and high TCE exposure based on concentrations of total trichloro compounds detected in urine	Duration of employment: 0.1 to 36.6 yr; average: 7 yr	Ambient air; occupational	Increases in high density lipoprotein cholesterol in the absence of elevation in plasma liver transaminases, indicating that low level exposure to TCE affects cholesterol metabolism without causing hepatocellular necrosis. Alcohol intake is an influential factor in the cross-sectional study.	Nagaya et al. 1993
Human workers (21 men, 1 woman)	Regular exposures of less than 5 ppm TCE; peak exposures for 2 workers at over 250 ppm	Mean duration of employment for TCE exposed workers: 7 yr	Ambient air; occupational	Highly significant increases in individual and total serum bile acids in the exposed group (controlled for age and alcohol intake). No abnormalities in liver function tests. No relationship between plasma bile acid and cholesterol was detected.	Driscoll et al. 1992
Human workers	8.9 + 3.1 ppm TCE	Mean duration of exposure: 3.4 yr	Ambient air; occupational	Elevation in total serum bile acids and some individual bile acids; normal hepatobiliary function tests.	Neghab et al. 1997

Abbreviation: TCE, trichloroethylene.

(NTP 2002b) study, male B6C3F$_1$ mice given chloral hydrate by gavage at 25, 50, or 100 mg/kg showed no significant changes in three serum liver transaminases, except for a significant increase in aspartate aminotransferase activity in the 50-mg/kg group.

Lash et al. (1995) investigated the toxicity of trichloroethylene and its metabolites with freshly isolated rat hepatocytes in culture. The studies showed that exposure to only *S*-(1,2-dichlorovinyl)-L-cysteine resulted in hepatocellular damage. None of the other metabolites (CYP450 dependant or reduced glutathione dependent) or trichloroethylene produced hepatocellular injury. The metabolites tested included trichloroacetic acid, dichloroacetic acid, chloral hydrate, trichloroethanol, oxalic acid, and *S*-1,2-dichlorovinyl-L-glutathione. Despite this lack of cytotoxicity, trichloroethylene and its metabolites produced mitochondrial dysfunction. This in vitro study showed that *S*-1,2-dichlorovinyl-L-cysteine is the only trichloroethylene-derived compound cytotoxic to rat hepatocytes in culture.

In contrast, in vivo studies showed that *S*-1,2-dichlorovinyl-L-cysteine has a very low hepatotoxic potential. Acute toxicity studies investigating the nephrotoxicity of *S*-1,2-dichlorovinyl-L-cysteine in male Swiss-Webster mice showed transient elevations in serum liver transaminases 12 hours after administration of the highest dose tested (75 mg/kg). This dose resulted in significant lethality due to nephrotoxicity at later times. No liver histopathology was detected at any of the doses or time points examined (Vaidya et al. 2003a).

Other studies investigating the epigenetic mechanisms of dichloroacetic-acid-induced carcinogenesis revealed morphologic evidence of liver injury that includes loss of cell membrane integrity, focal areas of cell debris, and appearance of apoptotic bodies in B6C3F$_1$ mice undergoing short-term exposure to dichloroacetic acid at 0.5 or 5 g/L for up to 30 days (Carter et al. 1995). Hepatocellular necrosis was also detected in male and female Swiss-Webster mice receiving dichloroacetic acid in their drinking water at 300, 1,000, or 2,000 mg/L for up to 14 days. This was accompanied by a marked increase in liver weights. No such changes were seen with trichloroacetic acid under the same dosing regimen (Bull et al. 1990; Sanchez and Bull 1990). Exposure of male B6C3F$_1$ mice to dichloroacetic acid (0.5 g/L) or chloral hydrate (1 g/L) via drinking water resulted in hepatocellular necrosis after 104 wk of exposure (Daniel et al. 1992).

Changes in Liver Glycogen Status

Exposure to trichloroethylene produces effects in the liver other than hepatocellular injury. Treating mice with dichloroacetic acid results in marked dose-dependent accumulation of liver glycogen (Bull et al. 1990; Kato-Weinstein et al. 1998). This dose-response relationship parallels that for the development of hepatocellular carcinomas. Furthermore, patients with glycogen storage disorders have a greater propensity for developing liver tumors (Labrune et al. 1997). These observations have prompted investigators to study in depth the relationship between increased liver glycogen storage and carcinogenesis.

Studies have been carried out to assess the effect of dichloroacetic acid treatment on insulin secretion, insulin receptor expression, and activity and expression of protein kinases controlled by insulin receptor signaling due to the role of these gene products in glycogen synthesis and homeostasis (Lingohr et al. 2001). Mice treated with dichloroacetic acid in drinking water at 0.1 to 2.0 g/L for 2-10 weeks showed a significant reduction in expression of the insulin receptor in the liver. As early as 2 weeks after the initiation of dichloroacetic acid

treatment, insulin concentrations were significantly reduced. Dichloroacetic acid similarly reduced the expression of protein kinase B (an insulin-sensitive enzyme involved in glycogen homeostasis). Because dichloroacetic-acid-induced glycogen accumulation precedes down-regulation of the insulin receptor and insulin-dependent signaling pathways, these changes in gene expression for insulin and related genes are considered to be compensatory responses to changes in glycogen homeostasis.

Lingohr et al. (2002) investigated whether the changes in glycogen accumulation brought about by dichloroacetic acid were insulin dependent. Freshly isolated mouse hepatocytes exposed to dichloroacetic acid accumulated more glycogen than control hepatocytes. This response was dose dependent. Omitting insulin from the culture media did not prevent the enhanced accumulation of glycogen produced by dichloroacetic acid. By contrast, dichloroacetic-acid-induced glycogen deposition was fully blocked by inhibition of the enzyme phosphatidylinositol 3-kinase. Phosphatidylinositol 3-kinase participates in the signal transduction pathway leading to glycogen synthesis initiated by activation of the insulin receptor. This suggests that dichloroacetic-acid-induced glycogen accumulation involves a phosphatidylinositol 3-kinase-dependent pathway downstream of the insulin receptor (Lingohr et al. 2002). In addition to regulating glycogen synthesis, phosphatidylinositol 3-kinase has been implicated in the proliferative and anti-apoptotic effects of peroxisome proliferators (Mounho and Thrall 1999), which further establishes an association between abnormal liver glycogen status and the carcinogenic effect of trichloroethylene and its metabolites. Other halogenated solvents such as bromochloroacetate and dibromoacetate induce glycogen accumulation in the liver to a similar degree as dichloroacetic acid (Kato-Weinstein et al. 2001).

Another potential link between aberrant liver glycogen homeostasis and the carcinogenicity of trichloroethylene is provided by the effect of this chemical and its metabolites on DNA methylation status. Exposure to dichloroacetic acid results in hypomethylation of protooncogenes and other genes involved in cell growth, such as insulin-like growth factor II in mouse liver (Tao et al. 2000a, 2004b; Ge et al. 2001). This response has been causally linked to the development of hepatocellular adenomas and carcinomas induced by these chemicals.

Dibromoacetic acid, a related haloacetic acid, similarly induced hepatic DNA hypomethylation in female mice and male rats receiving 1 or 2 g/L in their drinking water for up to 28 days. Specifically, hypomethylation of c-*myc* and insulin-like growth factor II was detected along with increases in mRNA expression of both genes in mouse liver. Only c-*myc* mRNA expression was increased in rat liver. Glycogen accumulation and induction of markers of peroxisome proliferation were also observed in mice and rats receiving dibromoacetic acid (Tao et al. 2004a). These results further support the relationship between glycogen accumulation and liver tumors induced by trichloroethylene and its metabolites, making it more uncertain whether trichloroethylene-induced glycogen accumulation can be considered a noncancer liver effect or an early event in carcinogenesis. More recently, Pereira et al. (2004) showed that methionine treatment not only prevents the DNA hypomethylation induced by dichloroacetic acid and related solvents, but it also prevents liver tumor formation in mice. Interestingly, methionine did not prevent glycogen accumulation completely. Dichloroacetic-acid-induced glycogen storage was reduced by only 25% with methionine treatment. This new information suggests dissociation between glycogen accumulation and the carcinogenic effects of dichloroacetic acid. Clearly, more studies are needed to clarify the relationship between altered glycogen storage and liver cancer in response to trichloroethylene.

Elevation of Serum Bile Acids

Occupational exposure to trichloroethylene has been reported to increase serum bile acid and cholesterol concentrations. Chronic occupational exposure to low concentrations of trichloroethylene appears to alter cholesterol metabolism in the absence of noticeable hepatocellular damage, as evidenced by lack of increase in serum liver transaminases (Nagaya et al. 1993). Similarly, serum concentrations of total and individual bile acids were significantly elevated in a group of workers exposed to trichloroethylene (Driscoll et al. 1992; Neghab et al. 1997). Because no association was observed between elevated plasma bile acids and conventional markers of liver injury, it was concluded that this perturbation in bile acid homeostasis could be indicative of early changes in liver function independent of hepatocellular damage.

Similar alterations in bile acid status have been observed in experimental animals exposed to trichloroethylene and its metabolites. Exposure to trichloroethylene via inhalation or intraperitoneal injection resulted in elevation of serum bile acid concentrations at doses that did not produce changes in markers of liver function, such as serum liver transaminases and bilirubin concentrations (Wang and Stacey 1990). To determine whether this increase in serum bile acids after low exposure to trichloroethylene is indicative of early liver dysfunction, the mechanism(s) responsible for these changes was investigated by examining the effect of trichloroethylene on bile acid transport in freshly isolated rat hepatocytes in culture (Bai and Stacey 1993). The uptake of the bile acids taurocholic and cholic acids into rat hepatocytes was inhibited in a dose-dependent manner by trichloroethylene at concentrations up to 1.84 mM. These concentrations did not result in significant leakage of transaminases or intracellular potassium into the culture media. Trichloroethylene inhibition of bile acids uptake was determined to be noncompetitive. These results indicate that the increase in serum bile acids produced by trichloroethylene occurs through interference with uptake into hepatocytes. The same study also determined that trichloroethylene does not affect the efflux of bile acids from hepatocytes. These observations clearly show that alterations in transport processes for bile acids (primarily by inhibiting uptake) occur in the absence of pathologic evidence of liver dysfunction.

The fact that inhibition of bile acid uptake by trichloroethylene was determined to be noncompetitive strongly argues against direct competition between trichloroethylene and bile acids, such as taurocholic and cholic acids, for the main basolateral carrier in hepatocytes for bile acids, the sodium taurocholate transporter polypeptide. This effect on bile acids is not unique for trichloroethylene, because occupational exposure to a mixture of other organic solvents including toluene, xylene, acetone, *n*-butanol, and ethylacetate similarly increases serum bile acids (Franco et al. 1986). These results also argue against competition for common transport as it is very unlikely that the uptake of all these solvents into hepatocytes requires the same transport process. Because of their high lipid solubility, these solvents can readily partition into the plasma membrane of hepatocytes by diffusion, which does not require transport protein function.

A decrease in hepatic bile acid uptake by trichloroethylene can be the result of changes in or disruption of plasma membrane lipids and changes in membrane fluidity. Changes in the fluidity of the plasma membrane lipid bilayer are known to affect the function of uptake transporters and other transmembrane proteins. Again, this is supported not only by the noncompetitive nature of the inhibition of the uptake of bile acids by trichloroethylene but also by its reversibility with time. In contrast, the lack of changes in bile acid efflux in trichloroethylene-exposed rat hepatocytes does not support the idea that changes in membrane

fluidity contribute to overall reduction in plasma membrane transport protein function, because changes in membrane fluidity should also affect the function of plasma membrane efflux transporters.

Earlier studies also demonstrated that 1,1,1-trichloroethane, a solvent similar to trichloroethylene, decreases ATP concentrations and inhibits the activity of plasma membrane ATPases in cultured rat hepatocytes in a dose-dependent manner. The only ultrastructural alteration detected in 1,1,1-trichloroethane-exposed hepatocytes was loss of membrane microvilli that was not associated with cell death (Kukongviriyapan et al. 1990). This reduction in ATP concentration could lead to impairment of energy-dependent transport processes across the plasma membrane of hepatocytes, thus providing a possible mechanistic explanation for the reduction in bile acid uptake into the liver of experimental animals. On the other hand, the main efflux transporter for bile acids in hepatocytes, known as the bile-acid-exporting pump, is also an ATP-dependent carrier. A reduction in cellular ATP quantities by 1,1,1-trichloroethane should also affect the efflux of bile acids from hepatocytes via the bile-acid-exporting pump if a reduction in cellular ATP status is a critical factor in the abnormal handling of bile acids with 1,1,1-trichloroethane exposure. However, this is not the case. Additional studies are required to better define the mechanisms by which trichloroethylene affects the vectorial transport of bile acids across hepatocytes.

The interference of 1,1,1-trichloroethane with the influx of chemicals into hepatocytes is not limited to bile acids because 1,1,1-trichloroethane also inhibited the uptake of ouabain and 2-aminoisobutyric acid (Kukongviriyapan et al. 1990). These two compounds are known to enter hepatocytes via an energy-dependent transport-mediated process. It is worth noting that the transport of cadmium chloride and 3-*O*-methyl-D-glucose does not change. Furthermore, occupational exposure to a mixture of organic solvents including toluene, xylene, acetone, *n*-butylacetate, *n*-butanol, and ethylacetate also results in elevation of mean serum bile acid concentrations in the absence of changes in biochemical markers of liver injury (Franco et al. 1986). Exposure to toluene by itself produces the same response (Neghab and Stacey 1997).

LIVER CANCER

This section provides an overview of the evidence on liver cancer caused by trichloroethylene from animal and human studies. Tables detailing dose-response data from animal studies are included to provide a context for assessing risks from environmental exposures and to conduct physiologically based pharmacokinetic modeling. The potential modes of action of carcinogenesis for trichloroethylene and its metabolites are then discussed.

Animal Studies

Trichloroethylene

Trichloroethylene induction of hepatic tumors in rodents was extensively reviewed by Bull et al. (2002). Gavage administration of trichloroethylene in corn oil has been shown to produce hepatic cancer in B6C3F$_1$ mice (NCI 1976; NTP 1990). One study (NCI 1976) used technical grade trichloroethylene (1,000 and 2,000 mg/kg for males, 700 and 1,400 mg/kg for

females) that contained 0.09% epichlorohydrin, a known rodent carcinogen. In another study (NTP 1990), a single dose of epichlorohydrin-free trichloroethylene was administered by gavage in corn oil to male and female B6C3F$_1$ mice at 1,000 mg/kg/day. Significant increases in the incidence of hepatocellular cancer were found in the mice.

Both pure amine-based-stabilized trichloroethylene and technical grade trichloroethylene with stabilizers (epichlorohydrin and 1,2-expoxybutane) were tested at 1.8 or 2.4 g/kg in corn oil in Swiss ICR/HA mice (Henschler et al. 1984). Neither compound produced liver tumors in the mice given daily doses for 18 months.

Stabilizer-free trichloroethylene was administered to male and female F344/N rats at 500 and 1,000 mg/kg for 103 wk (NTP 1990). Reduced survival was observed in male rats and a dose-related increase in hepatic cytomegaly occurred in both sexes. However, no hepatic adenomas or carcinomas were reported. Exposure to trichloroethylene likewise did not produce significant hepatic tumors in four other strains of rat of both sexes administered trichloroethylene by gavage in corn oil for 103 wk at doses from 125 to 2,000 mg/kg (NTP 1988).

Trichloracetic Acid

Trichloroacetic acid is a peroxisome proliferator and a species-specific carcinogen. As shown in Table 4-3, it induces hepatocellular carcinomas when administered in drinking water to male and female B6C3F$_1$ mice (a susceptible mouse strain). Dose-related increases in the incidence of malignant tumors and precancerous lesions have been observed with concentrations in drinking water up to 5 g/L. Significant increases in benign hyperplastic nodules and adenomas were found at concentrations in drinking water as low as 0.35 g/L. However, trichloroacetic acid has not induced significant hepatic tumors in male F344 rats under similar treatment conditions.

A large amount of trichloroacetic acid is formed in susceptible mouse strains after exposure to trichloroethylene (Green and Prout 1985), whereas only a minor portion is found in unresponsive strains of mice (Dekant et al. 1984, 1986b). Saturation of oxidative metabolism of trichloroethylene in rats results in trichloroacetic acid insufficient to induce peroxisome proliferation (Green 1990). Humans, like rats, may exhibit lower rates of oxidation and higher rates of conjugation than do mice.

The carcinogenic potential of peroxisome proliferators, such as trichloroacetic acid, in rodents might be associated with the ability of these agents to increase the rate of hepatocellular proliferation, resulting in hepatocellular hyperplasia and hepatomegaly (Marsman et al. 1988; Popp et al. 1994; Gonzalez et al. 1998). This mode-of-action is discussed later in this chapter.

Dichloroacetic Acid

Dichloroacetic acid, which is metabolized much more rapidly than trichloroacetic acid, is an effective inducer of hepatic tumors in mice and rats (see Table 4-4). It is a major metabolite of trichloroethylene in B6C3F$_1$ mice but is below the limit of detection in similarly dosed Sprague-Dawley rats (Larson and Bull 1992a). Hepatoproliferative lesions increased sharply in male B6C3F$_1$ mice when drinking water concentration increased from 1 to 2 g/L (Bull et al.

TABLE 4-3 Hepatocarcinogenic Effects of Trichloroacetic Acid in Drinking Water Studies with Mice and Rats

			Combined Hyperplastic Nodule and Hepatocellular Adenoma		Hepatocellular Carcinoma		
Species (sex)	Concentration (g/L)	Duration (wk)	Incidence	Tumor/n (multiplicity)	Incidence	Tumor/n (multiplicity)	Reference
$B6C3F_1$ mice (M)	0	61	2/22	0.09	0/22	0	Herren-Freund, et al.
	5	61	8/22	0.5	7/22	0.5	1987
$B6C3F_1$ mice (M)	0	52	1/35	0.03	0/35	0	Bull et al. 1990
	1	52	5/11	0.45	2/11	0.18	
	2	52	15/24	1.04	4/24	0.17	
	2	37	2/11	0.18	3/11	0.27	
$B6C3F_1$ mice (M)	0	60-95	Not reported	Not reported	6.7-10%	0.07-0.15	DeAngelo et al. 1991
	0.05	60	Not reported	Not reported	22%	0.31	
	0.5	60	Not reported	Not reported	38%	0.55	
	4.5	95	Not reported	Not reported	87%	2.2	
	5	60	Not reported	Not reported	55%	0.97	
$B6C3F_1$ mice (F)	0	52	1/40	0.03	0/40	0	Pereira 1996
	0.35	52	6/40	0.15	0/40	0	
	1.2	52	3/19	0.16	0/19	0	
	3.5	52	2/20	0.10	5/20	0.25	
	0	81	2/90	0.02	2/90	0.02	
	0.35	81	14/53	0	0/53	0	
	1.2	81	12/27	0	5/27	0	
	3.5	81	18/18	1.0	5/18	0.28	
F344 rats (M)	0	104	2/23	0.087	0/23	0	Daniel et al. 1993
	0.05	104	2/24	0.083	0/24	0	
	0.5	104	5/20	0.25	0/20	0	
	5	104	1/22	0.045	1/22	0.045	
F344/N rats (M)	0	104	0	0	0	0	DeAngelo et al. 1997
	0.05	104	0	0	0	0	
	0.5	104	0	0	0	0	
	5	104	0	0	0	0	

TABLE 4-4 Hepatocarcinogenic Effects of Dichloroacetic Acid in Drinking Water Studies with Mice and Rats

			Combined Hyperplastic Nodule and Hepatocellular Adenoma		Hepatocellular Carcinoma		
Species (sex)	Concentration (g/L)	Duration (wk)	Incidence	Tumor/n (multiplicity)	Incidence	Tumor/n (multiplicity	Reference
$B6C3F_1$ (M)	0	61					Herren-Freund et al. 1987
	5	61	25/26	4.6	21/26	1.7	
$B6C3F_1$ (M)	1	52	2/11	0.3	NR	NR	Bull et al. 1990
	2	52	23/24	3.6	5/24	0.25	
	2	37	7/11	2.2	0/11	0	
$B6C3F_1$ (M)	0	60	0/10	0	8/12	1.7	DeAngelo et al. 1999
	0.5	60			25/30	2.2	
	3.5	60	12/12	2.3			
	5	60	27/30	2.3			
	0	75	2/28	0.07			
	0.05	75	4/29	0.31			
	0.5	75	3/27	0.11			
	0	104	1/20	0.05	2/20	0.1	Daniel et al. 1992
	0.5	104	12/24	0.5	15/24	0.63	
$B6C3F_1$ (F)	0	52	1/40	0.03	0/40	0	Pereira 1996
	0.28	52	0/40	0	0/40	0	
	0.93	52	3/20	0.20	0/20	0	
	2.8	52	7/20	0.45	1/20	0.1	
	0	81	2/90	0.02	2/90	0.02	
	0.28	81	3/50	0.06	0/50	0	
	0.93	81	7/28	0.32	1/28	0.04	
	2.8	81	16/19	5.6	5/19	0.37	
$B6C3F_1$ (M)	0	100	14/50	0.25	5/50	0.28	DeAngelo et al. 1999
	0.05	100	11/33	0.5	NR	NR	
	0.5	100	11/24	0.32	5/24	0.68	
	1	100	23/32	0.8	16/32	1.29	
	2	100	13/14	0.85	6/14	2.47	
	3.5	100	8/8	0.64	4/8	2.9	
F344 (M)	0	60	0/7	0	0/7	0	DeAngelo et al. 1996
	0.05	60	0/7	0	0/7	0	
	0.5	60	0/7	0	0/7	0	
	2.4	60	26/27	0.96	1/27	0.04	
	0	104	1/23	0.04	0/23	0	
	0.05	104	0/26	0	0/26	0	
	0.5	104	9/29	0.31	2/29	0.1	
	2.4	104	Not done	Not done	Not done	Not done	

1990). Observations of greatly enlarged livers and marked cytomegaly in dichloroacetic-acid-treated mice led to the conclusion that tumorigenesis might depend largely on stimulation of cell division secondary to hepatotoxic damage. A follow-up histologic study to determine the dose relatedness of premalignant hepatic lesions was conducted (Carter et al. 2003) in liver tissues

from male B6C3F$_1$ mice treated in a study by DeAngelo et al. (1999). End points measured were altered hepatic foci, large foci of cellular alternations, adenomas, and carcinomas. Altered hepatic foci, large foci of cellular alterations, and adenomas demonstrated neoplastic progression with time; however, independent of dose and length of exposure, signs of toxicity were also observed in noninvolved liver. The authors interpreted these results as indicating that dichloroacetic acid behaves as a nongenotoxic carcinogen at doses below which genotoxicity has been observed.

In male F344 rats, dichloroacetic acid induced observable signs of toxicity in the nervous system, liver, and myocardium; however, treatment-related neoplastic lesions were observed only in the liver (DeAngelo et al. 1996). The low concentration of dichloroacetic acid in rats exposed to trichloroethylene may explain their relative insensitivity to hepatocarcinogenic effects. Dichloroacetic acid inhibits its own metabolism in the dose range 0.5 to 1 g/L in water; thus, sharp inconsistencies in blood concentrations are seen (ranging from <1 μM to 300-500 μM) (Kato-Weinstein et al. 1998) likely leading to some variation in tumor yield in the mouse and other findings studied in this dose range (Bull et al. 2002).

Chloral Hydrate

Chloral hydrate produces hepatic tumors in male B6C3F$_1$ mice but not in female B6C3F$_1$ mice or in F344 male rats (Table 4-5). A single dose of chloral hydrate at 10 mg/kg administered by intragastric intubation to mice at 15 days of age increased the number of tumors between 48 and 92 wk based on the appearance of three adenomas and three carcinomas among eight animals (Rijhsinghani et al. 1986). Chloral hydrate administered to male B6C3F$_1$ mice for 2 years in drinking water at an average dose of 166 mg/kg/day resulted in a 71% incidence of hepatic tumors (combined adenomas and carcinomas) (Daniel et al. 1992).

In another study, male B6C3F$_1$ mice and male F344/N rats were given chloral hydrate in drinking water for 2 years (George et al. 2000). Time-weighted mean daily doses in rats were 7.4, 37.4, and 162.6 mg/kg/day. No increase in prevalence (percentage of animals with a tumor) or multiplicity (tumors/animal) of hepatocellular tumors was seen in male rats. Time-weighted mean daily doses in mice were 13.5, 65.0, and 146.6 mg/kg/day. Water consumption, survival, and body and organ weights were not altered from control values in any of the chloral hydrate treatment groups for either species. It was concluded that chloral hydrate induced hepatocellular neoplasia in the mouse, with a significant increase in the prevalence of hepatoadenoma and multiplicity at all doses tested and a significant increase in hepatocellular carcinomas in the high-dose group.

A 2-year NTP study in female B6C3F$_1$ mice exposed to chloral hydrate administered in water by gavage was negative for induction of hepatic tumors up to a dose of 100 mg/kg (NTP 2002a). However, a 2-year NTP study in male B6C3F$_1$ mice at the same doses found some evidence of carcinogenic activity based on increased incidences of hepatocellular adenoma or carcinoma (combined) in mice fed ad libitum and increased incidences of hepatocellular carcinoma in dietary-controlled mice (NTP 2002a,b, 2003; Leakey et al. 2003a). Dietary-controlled mice received variably restricted feed allocations to maintain their body weight on a predetermined "idealized" weight curve predictive of a terminal background liver tumor incidence of 15% to 20%. A statistically significant dose response to chloral hydrate was observed in the dietary-controlled animals (terminally adjusted liver tumor incidence 23.4%,

TABLE 4-5 Hepatocarcinogenic Effects of Chloral Hydrate in Mice

Species (sex)	Dose (mg/kg)	Exposure Route/Vehicle	Duration (wk)	Combined Hyperplastic Nodule and Hepatocellular Adenoma		Hepatocellular Carcinoma		Reference
				Incidence	Tumor/n (multiplicity)	Incidence	Tumor/n (multiplicity)	
C57BL ×	0	Oral/drinking	92	0/19	0	2/19	0.11	Rijhsinghani
$C3HF_1$	5	water	92	2/9	0.22	1/9	0.11	et al. 1986
single dose	10		92	3/8	0.38	3/8	0.38	
to neonates								
$B6C3F_1$	0	Oral/drinking	104	1/20	0.05	2/20	0.10	Daniel et al.
	1	water	104	8/24	0.33	11/24	0.46	1992
$B6C3F_1$ (M)	0	Oral/drinking	104	21.4%	0.21	54.8%	0.74	George et al.
	13.5	water	104	43.5%	0.65	54.3%	0.72	2000
	65.0		104	51.3%	0.95	59.0%	1.03	
	146.6 mg/kg/day		104	50.0%	0.72	84.4%	0.72	
$B6C3F_1$ (F)	0	Oral	104	0/37	0	0	0	NTP 2002a
	25	gavage/distilled	104	0/48	0	0	0	
	50	water	104	0/43	0	0	0	
	100		104	0/36	0	0	0	
$B6C3F_1$ (M)	0	Oral	104	12/48	—	4/48	—	NTP 2002b
fed ad	25	gavage/distilled	104	19/48		10/48		
libitum	50	water	104	17/47		10/47		
	100		104	17/48		7/48		
$B6C3F_1$ (M)	0	Oral	104	—	—	16/48[a]	—	NTP 2002b
fed ad	25	gavage/distilled	104			25/48[a]		
libitum	50	water	104			23/47[a]		
	100		104			22/48[a]		
$B6C3F_1$ (M)	0	Oral	104	9/48	—	2/48	—	NTP 2002b;
controlled	25	gavage/distilled	104	7/48		5/48		Leakey et al.
dietary	50	water	104	10/48		4/48		2003a
intake	100		104	10/48		8/48		

[a]Adenoma or carcinoma.

23.9%, 29.7%, and 38.6% for the four dose groups) but not the test groups fed ad libitum (terminally adjusted liver tumor incidence 33.4%, 52.6%, 50.6%, and 46.2%) (Leakey et al. 2003b). Dietary control was deemed to improve survival and decrease interassay variation.

Overall, chloral hydrate appears to be a species-, strain-, and sex-specific weak carcinogen. Furthermore, because two of the metabolites of chloral hydrate are trichloroacetic acid and dichloroacetic acid, it is difficult to assess the direct contribution of chloral hydrate to the specific carcinogenic effects observed solely in male $B6C3F_1$ mice.

Collective Assessment of Animal Data

Trichloroacetic acid and chloral hydrate appear to be capable of inducing liver tumors only in mice, but dichloroacetic acid also induces liver tumors in rats. The blood concentration of trichloroacetic acid required to induce liver cancer in mice approaches the millimolar range; trichloroacetic acid is a peroxisome proliferator in the same dose range that induces liver cancer. The concentration of dichloroacetic acid associated with liver cancer is in the submicromolar range (Kalkuhl et al. 1998). The weak carcinogenicity of chloral hydrate is largely due to its metabolic conversion to trichloroacetic acid or dichloroacetic acid.

Altered gene expression in specific genes involved in the functional categories of cell growth, tissue remodeling, apoptosis, cancer progression, and xenobiotic metabolism has been observed in mouse liver after administration of a tumorigenic dose of dichloroacetic acid at 2 g/L in drinking water for 4 wk (Thai et al. 2003). Dichloroacetic acid produces tumors in mice that display immunoreactivity to a c-Jun antibody, whereas trichloroethylene-induced tumors do not show this antibody reactivity (Stauber and Bull 1997). More recent work, in which trichloroacetic acid and dichloroacetic acid were given to mice alone and in various dose combinations, showed that dichloroacetic acid and trichloroacetic acid produced some tumors that were c-Jun^+, and many that were c-Jun^-; the number with a mixed phenotype increased with the relative dose of dichloroacetic acid (Bull et al. 2002). Mutation frequency of the H-*ras* protooncogene in mouse tumors induced by trichloroacetic acid alone was significantly different from that observed in trichloroethylene-induced tumors (0.44 versus 0.21), but that observed with dichloroacetic-acid-induced tumors (0.33) was not significantly different from that observed with trichloroethylene. No significant difference was observed in mutation spectra of tumors produced by the three compounds. Thus, dichloroacetic acid appears to produce liver tumors with a different phenotype than those produced as a result of trichloroacetic acid exposure. Dichloroacetic acid also induces markedly enlarged livers associated with cytomegaly (Bull 2000).

Both trichloroacetic acid and dichloroacetic acid are effective as rodent liver carcinogens at doses that do not produce cytotoxicity. Trichloroacetic acid produces liver tumors in mice with a phenotype common to peroxisome proliferators, whereas dichloroacetic acid increases the growth of liver tumors and produces tumors with a phenotype distinct in several aspects from those produced by trichloroacetic acid. An initiation-promotion study of each compound alone or in pairwise combinations of trichloroacetic acid, dichloroacetic acid, and carbon tetrachloride was conducted in male $B6C3F_1$ mice (Bull et al. 2004). Carbon tetrachloride was chosen for its ability to promote growth of liver tumors through cytotoxicity, producing a reparative hyperplasia growth stimulus. Thus, trichloroacetic acid, dichloroacetic acid, and carbon tetrachloride were hypothesized to have individually different modes of action as promoters. In

general, interactions between carbon tetrachloride and trichloroacetic acid were seen to be additive and likely acting via different mechanisms whereas interactions between carbon tetrachloride and dichloroacetic acid were generally less than additive with a consistent dose-dependent decrease in the growth rate of tumors promoted by carbon tetrachloride. Dichloroacetic acid appears to exert an inhibitory effect on the growth of trichloroacetic-acid-promoted tumors. Thus, interactions were inhibitory or additive, but there appeared to be no evidence of synergy.

Differences between mice and rats in the development of hepatocellular adenoma and carcinoma from trichloroethylene and its metabolites is consistent with other non-mutagenic compounds and is not particularly useful for determination of mechanism of action or extrapolation to humans. This is due to controversy surrounding the validity of results in mouse liver resulting from the large number of non-mutagens that induce such tumors and the high and variable spontaneous tumor rates in some strains. Gold and Sloane (1995) examined the Carcinogenic Potency Database where 174 chemicals were evaluated as liver carcinogens in rats and mice. More mutagens than non-mutagens have been identified as liver carcinogens in each species (in mice 84 mutagens and 70 non-mutagens; in rats 75 mutagens and 32 non-mutagens). Their analysis indicated a species difference in the predominance of liver cancer in mice compared to rats. Among chemicals with positive results in the mouse, 55% (84/154) of mutagens compared to 71% (70/99) of non-mutagens induce liver tumors, while the proportions among positive chemicals in the rat are 39% (75/194) and 33% (32/98). Thus, while the proportion of rat carcinogens that are positive in the liver is similar for mutagens and non-mutagens, a higher proportion of non-mutagenic mouse carcinogens are positive in the liver than mutagenic carcinogens. This finding in mice reflects that chlorinated compounds (composed solely of chlorine, carbon, hydrogen, and, optionally, oxygen) are frequently positive in the mouse liver and are not mutagenic. Excluding the chlorinated compounds, results in mice are similar for mutagenic and non-mutagenic carcinogens; 56% (79/142) of mutagens and 59% (40/68) of non-mutagens are mouse liver carcinogens. In the Carcinogenic Potency Database, 261 rodent carcinogens have been tested in both rats and mice, and 82 (31%) induce tumors in only one target site of one species. The mouse liver is the most common single-site, single-species target organ for both mutagens (12 chemicals) and non-mutagens (19 chemicals). Many of the non-mutagens in this group are chlorinated compounds. Thus, the species difference in the potency of trichloroethylene and its metabolites to induce liver tumors must be put in context with this historical data.

Human Studies

Because it was not possible for the committee to provide a comprehensive evaluation of the epidemiologic evidence on trichloroethylene and different cancers, it borrowed a previously compiled summary of the epidemiologic evidence on liver cancer from the Institute of Medicine (IOM 2003) to give some perspective on the evidence for liver cancer (see Table 4-6). The list was updated with one study published since the IOM report. Some common limitations of the studies that were reviewed include a relatively small number of cases of liver cancer and a lack of control for potential confounding by risk factors such as alcohol consumption and hepatitis B (see methodology and exposure information on some of the specific studies reviewed in Chapter 3, Tables 3-4 and 3-6). Another issue is that some studies reported findings for primary liver

TABLE 4-6 Selected Epidemiologic Data on Liver Cancer or Hepatobiliary Cancers and Exposure to Trichloroethylene

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
Cohort Studies—Incidence			
Raaschou-Nielsen et al. 2003	Workers in Denmark		
	Males		
	All TCE-exposed workers	27[a]	1.1 (0.7-1.6)
	<1 yr employed	9	1.3 (0.6-2.5)
	1-4.9 yr employed	9	1.0 (0.5-1.9)
	≥5 yr employed	9	1.1 (0.5-2.1)
	Females		
	All TCE exposed workers	7	2.8 (1.1-5.8)
	<1 year employed	2	2.8 (0.3-10.0)
	1-4.9 yr employed	4	4.1 (1.1-10.5)
	≥5 yr employed	1	1.3 (0.0-7.1)
Morgan and Cassady 2002	Redlands, CA, community exposed to TCE in drinking water	28[a]	1.29 (99% CI 0.74-2.05)
Hansen et al. 2001	Biologically monitored Danish workers		
	Males	5[b]	2.6 (0.8-6.0)
	Females	0	—
Blair et al. 1998	Aircraft-maintenance workers in Utah		
	Males		
	No exposure	1[a]	0.8 (0.1-12.0)
	<5 unit-yr	2	1.2 (0.1-13.8)
	5-25 unit-yr	1	1.0 (0.1-16.0)
	>25 unit-yr	3	2.6 (0.3-25.0)
Antilla et al. 1995	Biologically monitored workers in Finland		
	Entire period since first measurement	5[a]	2.27 (0.74-5.29)
	0-9 yr	0	— (0.0-6.59)
	10-19 yr	2	1.74 (0.21-6.29)
	≥20 yr	3	6.07 (1.25-17.7)
	Mean personal U-TCA level		
	<100 μmol/L	2	1.64 (0.20-5.92)
	100+ μmol/L	2	2.74 (0.33-9.88)
Axelson et al. 1994	Biologically monitored Swedish workers	4[a]	1.41 (0.38-3)
Cohort Studies—Mortality			
Chang et al. 2003	Electronics-manufacturing workers in Taiwan[c]		
	Males	0	0.00 (NA)
	Females	0	0.00 (NA)
Boice et al. 1999	Aircraft-manufacturing workers in California		
	All exposed factory workers employed at least 1 yr since 1960 with routine exposure	4[b]	0.54 (0.15-1.38)
	Duration of potential exposure (routine or intermittent)		
	<1 year exposed	4[a]	0.53 (0.18-1.60)
	1-4 yr exposed	3	0.52 (0.15-1.79)
	≥5 yr exposed	6	0.94 (0.36-2.46)

TABLE 4-6 *Continued*

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% CI)
Ritz 1999	White male U.S. uranium-processing workers		
	TCE, cutting fluids, or kerosene	8[a]	1.66 (0.71-3.26)
	TCE, light exposure		
	>2 yr, no latency	3	0.93 (0.19-4.53)
	>2 yr, 15-yr latency	3	1.16 (0.24-5.60)
	>5 yr, no latency	3	1.90 (0.35-10.3)
	>5 yr, 15-yr latency	3	2.86 (0.48-17.3)
	TCE, moderate exposure		
	>2 yr, no latency	1	4.97 (0.48-51.1)
	>2 yr, 15-yr latency	1	5.53 (0.54-56.9)
	>5 yr, no latency	1	8.82 (0.79-98.6)
	>5 yr, 15-yr latency	1	12.1 (1.03-144)
Blair et al. 1998	Aircraft-maintenance workers in Utah		
	Primary liver cancer for all TCE exposed	4	1.7 (0.2-16.2)
	Liver and biliary cancer by cumulative TCE exposure		
	Males		
	No exposure	3	0.5 (0.1-2.4)
	<5 unit-yr	6	1.1 (0.3-4.1)
	5-25 yr	3	0.9 (0.2-4.3)
	>25 unit-yr	3	0.7 (0.2-3.2)
	Females		
	No exposure	3	4.2 (0.7-25.0)
	<5 unit-yr	1	1.6 (0.2-18.2)
	5-25 unit-yr	0	—
	>25 unit-yr	2	2.3 (0.3-16.7)
Morgan et al. 1998	Aerospace workers in Arizona		
	TCE-exposed subcohort:	6[b]	0.98 (0.36-2.13)
	Low cumulative exposure	3	1.32 (0.27-3.85)
	High cumulative exposure	3	0.78 (0.16-2.28)
	Peak and cumulative exposure[c]:		
	Peak: medium and high versus low and no exposure	3[a]	0.98 (0.29-3.35)
	Cumulative (low)	3	2.12 (0.59-7.66)
	Cumulative (high)	3	1.19 (0.34-4.16)
Greenland et al. 1994	White male transformer-assembly workers, ever exposed	NA	0.54 (0.11-2.63)
Garabrant et al. 1988	Aircraft-manufacturing workers, San Diego (about 37% of jobs had exposure to TCE)	8	0.94 (0.40-1.86)
Case-control—Mortality			
Lee et al. 2003	Community downstream of an electronics factory in Taiwan	53	2.57 (1.21-5.46)

[a]Results are for primary liver cancer.
[b]Results are for liver and biliary cancer combined.
[c]Internal cohort analyses for peak and cumulative exposure to trichloroethylene classifications used Cox proportional-hazards models.
Abbreviations: NA, not available; CI, confidence interval: TCE, trichloroethylene; U-TCA, urinary trichloroacetic acid.
Source: Adapted from IOM 2003.

cancer, and others reported findings for biliary and primary liver cancer combined. This could result in misclassification of the outcome if these two cancer sites (liver and biliary) are etiologically distinct with respect to the effects of trichloroethylene exposure. In addition, only large cohort studies would have adequate statistical power to estimate excess risks and exposure-response relationships, as the incidence of liver cancer in the United States is low; the age-adjusted rate of cancer of the liver and intrahepatic bile duct is 6 per 100,000 people (SEER 2005). The American Cancer Society (2006) estimates that approximately 18,500 people will be diagnosed liver and intrahepatic bile duct cancer in 2006.

Cohort Studies

Excess incidence of liver cancer was observed in most cohort studies that specifically examined exposures to trichloroethylene (Axelson et al. 1994; Antilla et al. 1995; Hansen et al. 2001; Morgan and Cassady 2002; Raaschou-Nielsen et al. 2003). These findings were generally based on a small number of incident cases and thus were statistically unstable. Only one study reported a statistically significant excess of liver cancer incidence for the entire cohort (Raaschou-Nielsen et al. 2003). An excess among women (relative risk [RR] = 2.8; 95% confidence interval [CI] = 1.1, 5.8), but not among males (RR = 1.1, 95% CI = 0.7, 1.6), was reported in this study. Liver cancer incidence among females was significantly increased among women with 1 to 4.9 years of exposure (RR = 4.1, 95%CI = 1.1, 10.5). The incidence of liver cancer was not significantly elevated in the highest exposure group (RR = 1.3, 95%CI = 0.0,7.1); however, there was only one case and less than one case expected in this group and, thus, the findings were highly unstable for this group. Evidence for an exposure-response relationship between the incidence of liver cancer and trichloroethylene exposure was also observed in the study by Antilla et al. (1995), who reported a statistically significant excess of liver cancer incidence in their highest duration of exposure category (>20 years; RR = 6.07, 95% CI = 1.25, 17.7).

Findings from the cohort studies that reported findings for mortality were mixed, with one study reporting no difference (Garabrant et al. 1988), three studies reporting a deficit (Greenland et al. 1994; Morgan et al. 1998; Boice et al. 1999), and two studies reporting an excess (Blair et al. 1998; Ritz 1999) in deaths from liver cancer. One study (Ritz 1999) found evidence of an exposure-response relationship; mortality from liver cancer was found to increase with degree (light versus moderate) and duration of exposure and time since first exposure (>15 years). A statistically significant excess of liver cancer (RR = 12.1) was reported among workers with moderate exposure, greater than 5 years of exposure, and at least 15 years since the first exposure; this finding was based on only one case and thus was not statistically stable (95% CI = 1.03, 144).

Case-Control Studies

A strength of the case-control studies is that they can have greater statistical power than cohort studies for evaluating rare outcomes such as liver cancer, but the power also depends on the prevalence of the exposure of interest, which is often low in general populations. A frequent weakness of population-based case-control studies is their inability to reliably document and

estimate workplace exposures. IOM (2003) identified four case-control studies of liver cancer and exposure to organic solvents in general (Stemhagen et al. 1983; Hardell et al. 1984; Hernberg et al. 1988; Heinemann et al. 2000). One study has been published since then in which exposure to trichloroethylene was investigated. Lee et al. (2003) conducted a population-based case-control study in a Taiwanese village downstream from an electronic factory that contaminated community wells with trichloroethylene, tetrachloroethylene, and 1,1-dichloroethylene. Trichloroethylene concentrations in the well water were reported to be an order of magnitude higher than those for tetrachloroethylene and 1,1-dichloroethylene. This study reported increased mortality odds ratios among males for all cancer and for liver cancer for the periods after 10 years of latency—namely, 1980-1989 and 1990-1997. The adjusted mortality odds ratios for liver cancer in males was 2.6 (95% CI = 1.2, 5.5), with a significant linear trend for the period effect. This study did not address potential confounding related to hepatitis viral infection status, a risk factor for liver cancer, or potential misclassification due to the inclusion of secondary liver cancer among the case series.

Mode of Action

A number of modes of action have been proposed for the carcinogenic action of trichloroethylene and its metabolites in the liver, including genotoxicity, mutagenicity activation of the nuclear receptor peroxisome proliferator-activated receptor α (PPARα), and alterations in cellular signaling pathways. This section reviews the available evidence for each of these modes of actions and the relevance to humans. Although these modes of action are discussed separately, it is likely that multiple modes of action are involved in the carcinogenic process.

Mutagenicity and Genotoxicity

Mutagenicity refers to the ability of a chemical to induce heritable mutations (damage that can pass to daughter somatic cells), whereas genotoxicity is a broader term that includes mutational end points, cytogenetic analysis, and primary DNA damage.

Most mutagenicity assays for trichloroacetic acid, dichloroacetic acid, and chloral hydrate are negative. Dichloroacetic acid and trichloroacetic acid do not consistently induce DNA damage in the livers of mice treated with hepatotoxic doses (IARC 1995a). The weight of evidence on the mutagenicity of chloral hydrate, dichloroacetic acid, and trichloroacetic acid indicates that a chemically induced mutation is unlikely to be a key event in the induction of tumors (Moore and Harrington-Brock 2000). In general, these chemicals require very high doses to elicit positive results, principally in in vitro tests. For example, chloral hydrate was positive in approximately 10 in vitro genotoxicity studies; however, in vivo results were mixed. Moreover, the potency of chloral hydrate in these studies was very low. Dichloroacetic acid has been the most extensively studied and has shown positive results in the standard Ames test protocol and in vitro mouse lymphoma assay; it was shown to induce very small increases in mouse bone marrow micronuclei and to increase DNA strand breaks in mouse and rat liver cells in vivo. However, DNA damage assays do not prove that a chemical can cause mutational damage. The collective evidence indicates that dichloroacetic acid is likely mutagenic but very weakly so. Trichloroacetic acid is the least mutagenic of the three metabolites, being negative in the

Salmonella test and only weakly positive in the mouse lymphoma assay. It is unlikely that trichloroacetic acid would contribute to tumor formation through a mutational mechanism.

Neonatal B6C3F$_1$ mice were administered chloral hydrate, trichloroacetic acid, and trichloroethylene by intraperitoneal injection at 8 and 15 days of age (Von Turgeln et al. 2002). At 12 months, only male mice treated with the positive control compounds had significant induction of liver tumors. Additional male mice were treated as above and livers were excised 24 and 48 hours and 7 days after the final dose. At 24 and 48 hours, mice treated with chloral hydrate or trichloroacetic acid showed significantly higher 8-oxo-2′deoxyguanosine formation, indicating increased endogenous DNA adduct formation through lipid peroxidation or oxidative stress; the authors concluded that neonatal B6C3F$_1$ male mice are not sensitive to chloral hydrate or trichloroacetic acid as liver carcinogens. DNA and insulin-like growth factor II were demonstrated to be hypomethylated in mouse liver tumors in an initiation-promotion experiment (Tao et al. 2004b). DNA in both dichloroacetic-acid- and trichloroacetic-acid-promoted tumors was shown to be hypomethylated. Specific genes involved in several functional categories, including cell growth, tissue remodeling, apoptosis, cancer progression, and xenobiotic metabolism, were shown to have altered gene expression in dichloroacetic-acid-induced mouse liver tumors (Thai et al. 2003). Overall, the evidence indicates that none of the three metabolites under consideration here is likely to act principally by a mutational or genotoxic mechanism as liver carcinogens.

Peroxisome Proliferator-Activated Receptor Agonism

Peroxisome proliferators are a class of compounds that when fed to laboratory animals result in liver cancer (see Appendix E for detail and perspective). A key mode of action in this carcinogenic process is activation of the nuclear receptor PPARα. The human relevance of PPAR agonism is a subject of debate in the scientific community that has resulted in at least two important working groups and subsequent publications (Klaunig et al. 2003; IAS 2005). Several review articles have detailed the role of peroxisome proliferators and PPARs in the carcinogenic process (Green 1992, 1995; Green and Wahli 1994; Cattley et al. 1995; James et al. 1998; Gelman et al. 1999; Vanden Heuvel 1999a,b; Bull 2000; Corton et al. 2000a,b; Yeldandi et al. 2000; Melnick 2001; Guan 2002; Youssef and Badr 2002, 2005; Yu et al. 2003; Zhao and Jiang 2003; Kennedy et al. 2004; Lai 2004; Bosgra et al. 2005; Corton and Lapinskas 2005; O'Brien et al. 2005). Trichloroethylene, trichloroacetic acid, and dichloroacetic acid are considered peroxisome proliferators and they induce morphologic and biochemical changes that typify this class of chemicals. Chloral hydrate is considered either a very weak or a nonperoxisome proliferator. Thus, at least in terms of trichloroethylene, trichloroacetic acid, and dichloroacetic acid, the PPARα agonism (i.e., peroxisome proliferation) mode of action is a viable possibility which will be examined in more detail herein. The general applicability of PPARα agonism to human health is discussed in Appendix E.

Peroxisome Proliferators and Liver Cancer

The proposed mode of action for peroxisome proliferators is depicted in Figure 4-1, which shows several key events that ultimately result in rodent liver tumors. First, peroxisome

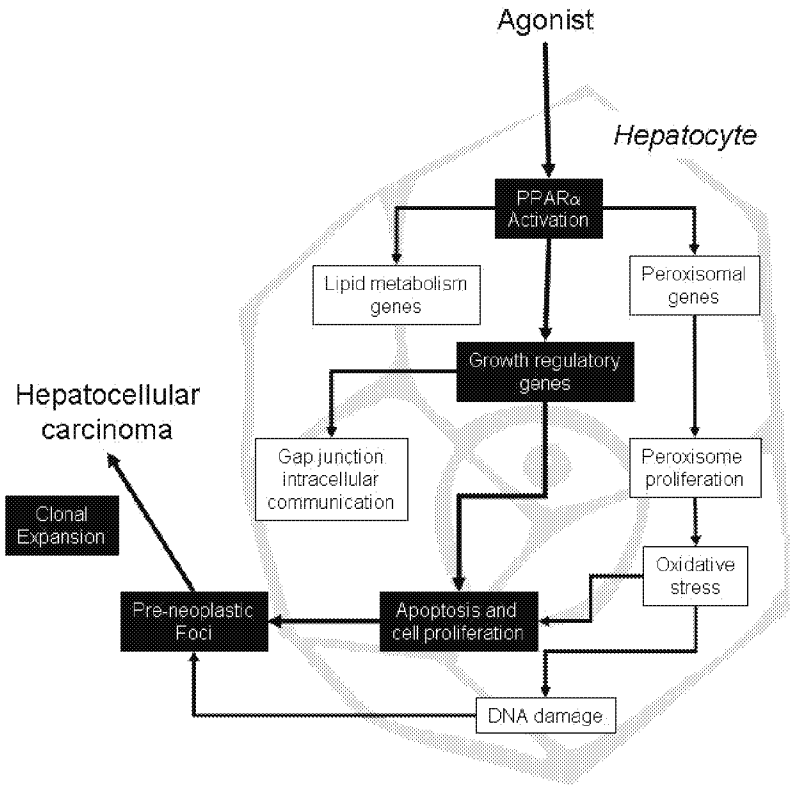

FIGURE 4-1 Proposed mode of action for liver tumor formation by peroxisome proliferators. Events causally related to adenoma or carcinoma formation are shown in black boxes; associated events are in white boxes. Source: Adapted from Klaunig et al. 2003.

proliferators activate PPARα, which regulates the transcription of genes involved in peroxisome proliferation, cell cycle and apoptosis, and lipid metabolism. Alterations in growth regulatory genes lead to perturbations in cell proliferation and apoptosis. Suppression of apoptosis coupled with stimulation of cell proliferation allows DNA-damaged cells to persist and proliferate, giving rise to preneoplastic foci and ultimately to tumors via further clonal expansion. Peroxisome proliferation per se is considered to be evidence of PPARα activation but it might or might not be related to the tumor formation. However, peroxisome proliferation could lead to oxidative stress, which could contribute to the mode of action by causing indirect DNA damage or by contributing to the stimulation of cell proliferation. As with other tumor promoters, PPARα ligands also inhibit gap junction intercellular communication, an event associated with increased cell proliferation. Several peroxisome proliferators stimulate nonparenchymal hepatic Kupffer cells and these resident macrophages affect the cell proliferation; this event is not depicted in Figure 4-1 because the importance of this event is controversial and no studies have been performed in this regard with trichloroethylene. The weight of evidence for the causally related events is discussed elsewhere (Klaunig et al. 2003; IAS 2005).

A minimal set of data elements to support a convincing demonstration that rodent liver tumors have arisen as a result of a PPARα mode of action would consist of PPARα agonism combined with light- or electron-microscopic evidence for peroxisome proliferation. Alternatively, evidence for PPARα agonism (in a receptor assay) combined with increases in

liver weight and one (induction of acyl CoA oxidase) or more (e.g., induction of CYP4A) of the specific in vivo markers of peroxisome proliferation would suffice. Demonstration that liver growth was accompanied by at least transiently increased rates of replicative DNA synthesis or decreased apoptosis also would significantly strengthen the case. The most convincing information showing that a particular compound induces liver cancer by PPARα mode of action would be through the use of the null mouse model. If the compound induces tumors in the wildtype, but not the PPARα$^{-/-}$ mouse, then this mode of action would be verified. Short of this information, the minimal criteria listed above (PPARα agonism with accompanying altered proliferation and growth characteristics) would be considered highly supportive. The absence of liver tumors in PPARα-null mice would definitively demonstrate the role of PPARα. Whether trichloroethylene and its metabolites meet this minimal data set is discussed below.

Trichloroethylene. The PPARα mode of action relative to trichloroethylene is summarized in Table 4-7; many of these effects might be attributable to trichloroethylene metabolites—in particular, trichloroacetic acid and dichloroacetic acid. Trichloroethylene activates mouse and human PPARα, albeit at high concentrations (1 mM), and can regulate known target genes for PPARα (Maloney and Waxman 1999; Nakajima et al. 2000; Laughter et al. 2004). Recent studies in PPARα null mice have shown that several events depend on this protein, including regulation of peroxisomal enzymes, cell proliferation, and perhaps some cell cycle regulatory genes (Klaunig et al. 1991; Stauber and Bull 1997; Tao et al. 1999, 2000b; Laughter et al. 2004). To the committee's knowledge, a long-term bioassay in this mouse model system has not been performed. An important characteristic of all tumor promoters is their ability to selectively enhance survival of a particular phenotype of foci that ultimately gains a growth advantage. The work of Stauber and Bull (1997) and Bull et al. (2002) examining oncogene expression and mutations as well as that Tao et al. (1999, 2000a,b) examining DNA methylation have provided substantial information on the tumor phenotypes in trichloroacetic-acid- and dichloroacetic-acid-treated rodents, although the data for trichloroethylene are much less extensive. However, trichloroethylene causes tumors that are mixed for c-*jun* expression but consistently contain codon 61 mutations in c-Ha-*ras*. Interestingly, the tumor phenotypes of trichloroethylene-, trichloroacetic-acid, and dichloroacetic-acid-induced tumors are not identical.

The species difference in tumor-promoting effects between rats and mice can be examined relative to the PPARα mode of action. The species difference in sensitivity to palmitoyl CoA oxidation activity was studied in F344 rats after treatment with trichloroethylene, tetrachloroethylene, and trichloroacetic acid (Goldsworthy and Popp 1987), and in Osborne-Mendel and Alderly Park rats and B6C3F$_1$ and Alderley Park mice treated with trichloroethylene (Elcombe et al. 1985). The data indicate that rats treated with trichloroethylene (or tetrachloroethylene) do not show increases in peroxisomal enzyme activities, whereas rats treated with trichloroacetic acid have significant increases in peroxisomal enzyme activity. On the other hand, mice responded to treatment with trichloroethylene, exhibiting increases in peroxisomal volume density and induction of peroxisomal enzyme activities, catalase, and palmitoyl CoA oxidation. The accepted explanation for this species difference in sensitivity is a difference in metabolism. Trichloroethylene is metabolized by cytochrome P-450s and other noncytochrome P-450 oxidative enzymes to trichloroacetic acid and dichloroacetic acid. Goldsworthy and Popp (1987) clearly demonstrated that trichloroacetic acid was a peroxisome proliferator in rats. Yet rats metabolize trichloroethylene more slowly than mice and metabolism appears to be saturable

TABLE 4-7 Trichloroethylene and PPARα Mode of Action

Event	Comments	References
Causal Events		
PPARα activation	Human and mouse PPARα activated in transient transfection reporter assays. Studies from PPARα null mice show that the effects on cell proliferation and peroxisome proliferator target genes are PPARα dependent.	Maloney and Waxman 1999; Nakajima et al. 2000; Laughter et al. 2004
Regulation of growth regulatory genes	Increased c-jun and c-myc mRNA levels in nontumor tissue. Several potential growth regulatory target genes examined using microarrays showing a PPARα-dependent response.	Tao et al. 1999, 2000b; Laughter et al. 2004
Cell proliferation or apoptosis	Although there is no or little increase in hepatocyte labeling index in rats, mice exposed to trichloroethylene have higher rates of cell proliferation. This event is PPARα dependent. Trichloroethylene inhibited intercellular communication in mouse hepatocytes and not in rat hepatocytes.	Klaunig et al. 1989; 1991; Stauber and Bull 1997; Laughter et al. 2004
Clonal expansion	Tumors that arise from trichloroethylene treatment are basophilic with a relatively consistent mutational spectrum (c-Ha-*ras* codon 61).	Bull et al. 2002
Associative Events		
Peroxisome proliferation and regulation of lipid metabolism genes	Increased peroxisomes and peroxisomal enzymes are seen in mice but much less in rats. Increases in CN-insensitive palmitoyl CoA oxidation is induced by trichloroethylene but to a lesser extent than fibrates. ACO and CYP4A induction is PPARα dependent.	Goldsworthy and Popp 1987; NTP 1988; Nakajima et al. 2000; Laughter et al. 2004
Oxidative stress	Increased thiobarbituric acid reactive substances and decreases in reduced glutathione in mouse liver.	Watanabe and Fukui 2000

(Green and Prout 1985; Prout et al. 1985; Green et al. 1997a). Thus, the lack of tumorigenesis in rats compared with mice can be explained by a difference in metabolism of the active metabolite.

Trichloroacetic Acid. Trichloroacetic acid is most often cited as the hepatocarcinogenic metabolite of trichloroethylene. Thus, much of the same evidence for a PPARα mode of action for trichloroethylene could be provided for trichloroacetic acid. However, as outlined in Table 4-8, there are some differences in the strength of the data relative to trichloroethylene. There have been no studies showing the PPARα dependence on cell proliferation induced by trichloroethylene, although hypertrophy was not seen in PPARα null mice. A more extensive characterization of trichloroacetic-acid-induced tumors is available that clearly shows clonal

TABLE 4-8 Trichloroacetic Acid and PPARα Mode of Action

Event	Comments	References
Causal Events		
PPARα activation	Mouse PPARα activated in transient transfection reporter assays with mixed results regarding human PPARα. Studies from PPARα null mice show that effects on peroxisome proliferator target genes are PPARα dependent.	Maloney and Waxman 1999; Walgren et al. 2000a,b; Laughter et al. 2004
Regulation of growth regulatory genes	Has not been studied.	
Cell proliferation or apoptosis	Increased labeling index and dose-dependent increases in cell proliferation were observed. Hypertrophy was noted in the PPARα wild-type but not PPARα null mice. Trichloroacetic acid inhibited intercellular communication in mouse hepatocytes but not in rate hepatocytes.	Stauber and Bull 1997; Klaunig et al. 1989; Ge et al. 2001; Laughter et al. 2004
Clonal expansion	Increased clonal expansion of tumors that resemble those seen by peroxisome proliferators (and unlike DCA). Basophilic foci lacking GST-pi. See spectrum of mutations in K- and H-*ras*. Expansion not due to cytotoxicity. DNA hypomethylation is seen in TCA-induced tumors in the promoters of c-*myc*, IGF-II, and c-*jun*.	Ferreira-Gonzalez et al. 1995; Pereira 1996; Pereira and Phelps 1996; Pereira et al. 1997, 2001; Tao et al. 1996, 2004b; Latendresse and Pereira 1997; Bull et al. 2004
Associative Events		
Peroxisome proliferation and regulation of lipid metabolism genes	Increases in peroxisomal enzymes are seen in mouse and to a lesser extent in rat liver. CYP4A induction is PPARα dependent.	Goldsworthy and Popp 1987; Odum et al. 1988; Walgren et al. 2004
Oxidative stress	Has not been studied.	

Abbreviations: DCA, dichloroacetic acid; GST, glutathione *S*-transferase; IGF-II, insulin-like growth factor II; TCA, trichloroacetic acid.

expansion occurs as a result of treatment with this chemical (greater evidence than provided by trichloroethylene).

Dichloroacetic Acid. Although the initial events in the PPARα mode of action associated with dichloroacetic acid seem similar to those of trichloroethylene and trichloroacetic acid, there are some intriguing differences (see Table 4-9). For example, although dichloroacetic acid can activate PPARα (perhaps slightly less than trichloroacetic acid does) and cause peroxisome proliferation, the liver hypertrophy is not PPARα dependent. Also, the clonal expansion of preneoplastic foci by dichloroacetic acid is quite different with different phenotypic and genetic markers than by trichloroacetic acid and trichloroethylene.

Chloral Hydrate. Although chloral hydrate is used medically as a sedative or hypnotic and as a rubefacient in topical preparations, it has not been studied extensively in terms of the PPARα mode of action (see Table 4-10). The most extensive study was performed by the

TABLE 4-9 Dichloroacetic Acid and PPARα Mode of Action

Event	Comments	References
Causal Events		
PPARα activation	Mouse PPARα activated in transient transfection reporter assays with mixed results regarding human PPARα. Studies from PPARα null mice show that the effects on peroxisome proliferator target genes are PPARα dependent.	Maloney and Waxman 1999; Walgren et al. 2000a,b; Laughter et al. 2004
Regulation of growth regulatory genes	Has not been studied.	
Cell proliferation or apoptosis	Increased labeling index and dose-dependent increases in cell proliferation were observed. Hypertrophy was noted in the PPARα wild-type and PPARα null mice.	Stauber and Bull 1997; DeAngelo et al. 1999; Walgren 2000a,b; Ge et al. 2001; Laughter et al. 2004
Clonal expansion	Increased clonal expansion of tumors that do not resemble those seen by peroxisome proliferators (and unlike TCA and trichloroethylene). Eosinophilic foci positive for GST-pi, TGF-α, c-*jun*, and c-*myc* and negative for c-*fos*. DNA hypomethylation is seen in DCA-induced tumors in the promoters of c-*myc* and IGF-II. This hypomethylation and tumor formation can be reversed by methionine (although peroxisome proliferation may not be). Reversal of hypomethylation can be reversed after removal of DCA (unlike TCA). Loss of heterozygosity is observed in DCA-induced tumors.	Anna et al. 1994; Ferreira-Gonzalez et al. 1995; Pereira 1996; Pereira and Phelps 1996; Pereira et al. 1997, 2004; Tao et al. 1996, 2004b; Latendresse and Pereira 1997; Miller et al. 2000; Carter et al. 2003
Associative Events		
Peroxisome proliferation and regulation of lipid metabolism genes	Increases in peroxisomal enzymes are seen in mouse and to a lesser extent in rat liver. CYP4A induction is PPARα dependent.	Everhart et al. 1998; DeAngelo et al. 1999; Pereira et al. 2004; Walgren 2004

Abbreviations: DCA, dichloroacetic acid; GST, glutathione *S*-transferase; IGF-II, insulin-like growth factor II; TCA, trichloroacetic acid; TGF-α, transforming growth factor type α.

National Toxicology Program (NTP 2002b). Groups of male mice received chloral hydrate in distilled water by gavage at doses of 25, 50, or 100 mg/kg 5 days per week for 104 to 105 wk. Each dose group was divided into two dietary groups of mice. The mice fed ad libitum had free access to feed, and the diet-controlled mice received feed in measured daily amounts calculated to maintain body weight on a previously computed idealized body weight curve. Chloral hydrate did not significantly induce either lauric acid 4-hydroxylase activity or CYP4A immunoreactive protein in any of the dosed groups of mice fed ad libitum. However, the high dose significantly induced both lauric acid 4-hydroxylase activity and CYP4A immunoreactive protein in the diet-controlled mice. Moreover, the induction-response profile of CYP4A was similar to the increase in the incidence of liver neoplasms at 2 years in the diet-controlled mice, with the major effect occurring in the 100-mg/kg group.

Weight of Evidence

Table 4-11 summarizes the committee's evaluation of the weight of evidence for a PPARα mode of action in rodents for trichloroethylene and its metabolites. The assessment is based on the general framework described by Cohen et al. (2003) and illustrated for PPARα agonists by Klaunig et al. (2003). Briefly, strong weight of evidence is defined as several studies that support the mode of action, and weak weight of evidence is defined by having a single study from a single laboratory or a significant amount of contradiction in the literature.

Dose-Response

The dose-response relationships for the key events in the PPARα mode of action are shown in the Table 4-12. Note the results from the comparison of wild-type with PPARα null mice that show the role of this receptor in the toxicity of trichloroethylene or its metabolites (discussed earlier in this chapter). PPARα activation per se is reserved for trans-activation assays, as that is the most direct and definitive way to examine nuclear receptor agonism.

TABLE 4-10 Chloral Hydrate and PPARα Mode of Action

Event	Comments	References
Causal Events		
PPARα activation	Has not been studied.	
Regulation of growth regulatory genes	Has not been studied.	
Cell proliferation or apoptosis	Although hepatocelluar carcinoma was observed in mice and not rats, there was no increase in cell proliferation in either species. Chloral hydrate had no effect on hepatocyte intercellular communication in either rat or mouse cells.	Klaunig et al. 1989; George et al 2000
Clonal expansion	Has not been studied.	
Associative Events		
Peroxisome proliferation and regulation of lipid metabolism genes	Although hepatocellular carcinoma was observed in mice and not rats, there was no increase in palmitoyl CoA oxidation in either species. In diet-controlled mice, peroxisome proliferation and an increase in CYP4A protein and enzyme activity were seen.	George et al 2000; NTP 2002b
Oxidative stress	Has not been studied.	

TABLE 4-11 Strength of the Weight of Evidence for PPARα Mode of Action for Trichloroethylene and Its Metabolites

Chemical	Weight of Evidence[a]	Comments
TCE	Strong	TCE activates PPARα at high concentrations and regulates a variety of target genes. Studies with PPARα null mice show that most responses are dependent on this receptor, including peroxisome proliferation, cell proliferation, and target gene expression. More evidence could be provided by a long-term bioassay in this model system.
TCA	Strong	TCA activates PPARα at high concentrations. Less extensive characterization of PPARα dependence on cell proliferation is provided than is known for TCE. However, the evidence of clonal expansion and phenotypic characteristics of tumors is strong and shows similarity to peroxisome proliferators.
DCA	Strong	DCA activates PPARα at high concentrations. As is the case with TCA, PPARα dependence of DCA's effects on cell proliferation is less than for trichloroethylene. Increasingly, it appears that DCA-induced clonal expansion is dissimilar to that of TCA and TCE. The reason for this discrepancy is not known but may require examination of tumors from PPARα null mice. Also, liver weight changes (and presumably cell proliferation) are not dependent on PPARα, indicating a potential for other modes of action that would be different than that of TCA.
Chloral hydrate	Weak	There is no evidence of PPARα activation by chloral hydrate aside from it being a weak peroxisome proliferator.

[a]A strong weight of evidence is defined as evidence from several studies which support the mode of action, while a weak weight of evidence is defined as having a single study from a single laboratory or a significant amount of contradiction in the literature (Klaunig et al. 2003).
Abbreviations: DCA, dichloroacetic acid; TCA, trichloroacetic acid; TCE, trichloroethylene.

FINDINGS AND RECOMMEDATIONS

Hepatotoxicity

The existing data clearly demonstrate that trichloroethylene produces hepatotoxicity in experimental animals and humans that is dependent on generation of reactive intermediates by CYP-450 in the liver. Besides its hepatotoxic potential, trichloroethylene and its metabolites produce liver effects categorized as independent of hepatotoxicity. These effects include elevations in plasma bile acids and accumulation of liver glycogen in the absence of subclinical evidence of liver dysfunction. The absence of liver dysfunction in rodents has been documented in studies using serum markers of liver injury and is further supported by histopathologic examinations that showed no ultrastructural changes. However, the absence of liver dysfunction in humans has been based entirely on measures of serum markers of liver injury (e.g., plasma transaminases, bilirubin concentrations). Therefore, the possibility that humans might have discrete ultrastructural changes in the liver that can affect bile acid homeostasis cannot be ruled

TABLE 4-12 PPARα Mode-of-Action Dose-Response Relationships

Effect	Dose/Concentration[a]	Route	Vehicle	Duration	Gender	Species/Strain	Reference
Causal Events							
PPARα Activation							
	TCA 1.0 mM; 5 mM	In vitro	DMSO	24 hr	N/A	Cos-1 cells transfected with human and mouse PPARα	Maloney and Waxman 1999
	DCA, 1.0 mM, 5 mM	In vitro	DMSO	24 hr	N/A	Cos-1 cells transfected with human and mouse PPARα	Maloney and Waxman 1999
	TCA, 4 mM (DCA at 4 mM, no effect)	In vitro	Unknown	24 hr	N/A	HL8.5 cells transfected with mouse PPARα	Walgren et al. 2000b
Regulation of Growth Regulatory Genes							
c-*jun*, c-*myc*	TCE 1000 mg/kg	Oral gavage, 5 days/wk	Corn oil	33 days	Female	$B6C3F_1$ mice	Tao et al. 1999
c-*jun*, c-*myc* (in tumors)	DCA, 20 mmol/L	Drinking water	Water	46 wk	Female	$B6C3F_1$ mice	Tao et al. 1999
c-*jun*, c-*myc* (in tumors)	TCA 20 mmol/L	Drinking water	Water	46 wk	Female	$B6C3F_1$ mice	Tao et al. 1999
c-*jun*, c-*myc*	TCE, 1000 mg/kg	Oral gavage	Corn oil	5 days	Female	$B6C3F_1$ mice	Tao et al. 2000b
c-*jun*, c-*myc*	TCA, 500 mg/kg	Oral gavage	Water	5 days	Female	$B6C3F_1$ mice	Tao et al. 2000b
c-*jun*, c-*myc*	DCA, 500 mg/kg	Oral gavage	Water	5 days	Female	$B6C3F_1$ mice	Tao et al. 2000b
Growth regulatory genes via microarray	TCE, 1500 mg/kg	Oral gavage	Methyl cellulose (0.1%)	3 days	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004
Cell Proliferation or Apoptosis							
BrdU labeling	TCE, 500 and 1,000 mg/kg/day (not observed in PPARα null)	Oral gavage	Methyl cellulose (0.1%)	3 wk	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004
[^{3}H]Thymidine incorporation	TCE, 500 mg/kg	Oral gavage	Corn oil	7, 14 days	Male	$B6C3F_1$ mice	Klaunig et al. 1991

TABLE 4-12 *Continued*

Effect	Dose/Concentration[a]	Route	Vehicle	Duration	Gender	Species/Strain	Reference
[^{3}H]Thymidine incorporation	TCE, no effect	Oral gavage	Corn oil	3, 7, 14 days	Female	B6C3F$_1$ mice	Klaunig et al. 1991
[^{3}H]Thymidine incorporation	TCE, no effect	Oral gavage	Corn oil	3, 7, 14 days	Male	F344 rat	Klaunig et al. 1991
[^{3}H]Thymidine incorporation	TCE, no effect	Oral gavage	Corn oil	3, 7, 14 days	Male	F344 rat	Klaunig et al. 1991
BrdU labeling (not within tumors)	DCA, 2 g/L	Drinking water	Water	14 days	Male	B6C3F$_1$ mice	Stauber and Bull 1997
BrdU labeling (not within tumors)	TCA, 2 g/L	Drinking water	Water	14, 28 days	Male	B6C3F$_1$ mice	Stauber and Bull 1997
[^{3}H]Thymidine incorporation	TCE, 250-2,500 mg/kg	Intraperitoneal	Corn Oil	24 hr (500, 1,250) 36 hr (250-1,250) 48 hr (all doses) 72 hr (250, 1,250, 2,500) 96 hr (none)	Male	Sprague-Dawley rats	Soni et al. 1998
Anchorage-independent growth	TCA, DCA, 0.5-2 mM	In vitro	Media	10-25 days	Male	Hepatocytes from B6C3F$_1$ mice	Stauber et al. 1998
[^{3}H]Thymidine incorporation	TCA, DCA 0.1-1 mM. Varied based on individual	In vitro	Media	72 hr	Male and Female	Human primary cultures	Walgren et al. 2000a
PCNA labeling	TCA, DCA 500 mg/kg	Oral gavage	Saline	72-96 hr	Female	B6C3F$_1$ mice	Ge et al. 2001
Clonal Expansion[b]							
Associative Events							
Peroxisome Proliferation and Regulation of Lipid Metabolism Genes							
CYP 4a12 mRNA	TCE, 1,500 mg/kg/day (not observed in PPARα null)	Oral gavage	Methyl cellulose (0.1%)	3 days	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004
CYP4A, ACO protein,	TCE, 125, 500, 1,000 mg/kg/day (not observed in PPARα null)	Oral gavage	Methyl cellulose (0.1%)	3 wk	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004

TABLE 4-12 *Continued*

Effect	Dose/Concentration[a]	Route	Vehicle	Duration	Gender	Species/Strain	Reference
Palmitoyl CoA oxidase	TCE, 1,500 mg/kg/day (not observed in PPARα null)	Oral gavage	Methyl cellulose (0.1%)	3 wk	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004
CYP4A, ACO protein,	TCA, 1.0, 2.0 M (not observed in PPARα null)	Drinking water	Water	1 week	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004
Palmitoyl CoA oxidase	TCA, 2 M (not observed in PPARα null)	Drinking water	Water	3 wk	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004
CYP4A protein	DCA, 2 M (not observed in PPARα null)	Drinking water	Water	3 wk	Male	SV129 wild-type and PPARα null mice	Laughter et al. 2004
Palmitoyl CoA oxidase	TCA, DCA 600 mg/kg	In vitro	Media	24 hr	Male and Female	$B6C3F_1$ mouse liver homogenate and primary culture. No effects seen with LEH rat or human cells/cultures	Walgren et al. 2000a
Laural CoA oxidase	DCA, 3.2 g/l Unaffected by methionine	Drinking water	Water	8 and 44 wk	Female	$B6C3F_1$ mice	Pereira et al. 2004
Lauric acid oxidase; CYP4A protein	CH, 100 mg/kg	Gavage (5 days/week)	Water	104 wk	Male	$B6C3F_1$/Nctr mice	Leakey et al. 2003a
Palmitoyl CoA oxidase activity	TCA, 5 g/L MCA, no effect	Drinking water	Water	15-104 wk	Male	F344 rats	DeAngelo et al. 1997
Palmitoyl CoA oxidase activity	Perc, 200, 400 ppm	Inhalation	Air	28 days	Male and Female	F344 rats $B6CF_1$ mice	Odum et al. 1988
Peroxisome number	Perc, 200, 400 ppm	Inhalation	Air	28 days	Male and Female	$B6CF_1$ mice	Odum et al. 1988
Palmitoyl CoA oxidase activity	MCA, 500 μM DCA, 1000 μM TCA, 500 μM	In vitro	Media	72 hr	Male	Long Evans rat primary hepatocytes	Walgren et al. 2004
Peroxisome proliferation	TCE, 0.75 g/kg	Gavage (daily)	Corn oil	2 wk	Male and Female	Sv/129 (not seen in PPARα null)	Nakajima et al. 2000

TABLE 4-12 *Continued*

Effect	Dose/Concentration[a]	Route	Vehicle	Duration	Gender	Species/Strain	Reference
Peroxisome proliferation	TCE, 0.75 g/kg	Gavage (daily)	Corn oil	2 wk	Male	Sv/129 (not seen in PPARα null or in female wt or null)	Nakajima et al. 2000
Palmitoyl CoA oxidase activity	DCA, 0.5 mM (mouse); 1.0 mM (rat)	In vitro	Media	72 hr	Male	$B6C3F_1$ mice, Long Evans rat primary hepatocytes	Everhart et al. 1998
Palmitoyl CoA oxidase activity	TCE, 100 mg/kg TCA, 500 mg/kg Perc, 1,000 mg/kg (¼- ½ the effect seen with Wy14,643 at 50 mg/kg)	Gavage (10 days)	DMSO/corn oil	10 days	Male	$B6C3F_1$ mice, F344 rats	Goldsworthy and Popp 1987
Oxidative stress[c]							

[a]Doses where statistically significant effects were observed.
[b]Clonal expansion is pertinent only in reference to tumor formation and phenotyping of foci and nodules.
[c]Signs of oxidative stress, including glycogen accumulation.
Abbreviations: BrdU, bromodeoxyuridine; CH, chloral hydrate; DCA, dichloroacetic acid; DMSO, dimethyl sulfoxide; MCA, monochloroacetic acid; Perc, tetrachloroethylene; TCA, trichloroacetic acid; TCE, trichloroethylene.

out. There are some mechanistic data addressing the nature of the elevation in bile acids in plasma but the precise mode of action remains unknown.

Elevation of plasma bile acids could result in their accumulation in other tissues, which could conceivably have detrimental effects on those organs. In addition to their lipid-solubilizing effect, bile acids are also signaling molecules that regulate gene transcription. The farnesoid X receptor functions as a bile acid nuclear receptor which regulates transcription of multiple genes responsible for maintaining cholesterol and bile acid homeostasis. Thus, accumulation of bile acids might contribute to the adverse effects of trichloroethylene exposure in organs other than the liver through a detergent effect or altered cellular signaling. However, it is not clear whether or not this is a significant effect.

The human relevance of liver glycogen accumulation observed in rodents exposed to dichloroacetic acid remains unclear. There are no studies documenting this effect in humans. Furthermore, all research on glycogen accumulation has been carried out using dichloroacetic acid, and not trichloroethylene. In light of this, it is not known whether exposure to trichloroethylene at environmentally relevant concentrations results in glycogen accumulation in rodents or humans.

Investigators have been able to dissociate the glycogen deposition effect from the peroxisome proliferation produced by trichloroethylene and its metabolites because dichloroacetic acid, which produces significant liver enlargement and no peroxisome proliferation, induces a marked accumulation of liver glycogen. In contrast, exposure to trichloroacetic acid produces only modest glycogen accumulation while stimulating considerable peroxisome proliferation.

Data from studies with autoimmune-prone mice also suggest that trichloroethylene and its metabolites are capable of triggering an immune-mediated reaction against the liver. This observation is highly relevant to humans because there are multiple case reports of workers exposed to trichloroethylene with Stevens-Johnson syndrome who developed generalized skin reactions often accompanied by hepatitis of acute onset.

In summary, data generated since EPA (2001b) released its draft health risk assessment have not significantly advanced understanding of whether some of the noncancer liver effects of trichloroethylene and its metabolites are independent of early ultrastructural and discrete pathologic changes. Also, the relationship of these effects to hepatocarcinogenesis remains unclear.

Liver Cancer

Data on trichloroethylene indicate that relatively high doses are needed to induce liver cancer, even in susceptible strains of mice. The three major metabolites of trichloroethylene—trichloroacetic acid, dichloroacetic acid, and chloral hydrate—can contribute to liver cancer in mice. None of the three is directly mutagenic or genotoxic as the principal mode of action. Trichloroacetic acid and dichloroacetic acid have been shown to promote liver cancer in classic initiation-promotion experimental protocols. The concentrations of trichloroacetic acid in blood required to induce liver cancer approach the millimolar range, whereas dichloroacetic acid concentrations in blood associated with carcinogenesis are in the submicromolar range. The carcinogenic activity of chloral hydrate is largely dependent on its conversion to trichloroacetic

acid and dichloroacetic acid. Dichloroacetic acid and trichloroacetic acid adequately account for the hepatocarcinogenic responses to trichloroethylene.

There is sufficient weight of evidence to conclude that the mode of action of trichloroacetic acid as a rodent liver carcinogen is principally as a liver peroxisome proliferator in a specific strain of mouse, $B6C3F_1$. This strain also has a particularly high background incidence of liver tumors. Moreover, F344 rats in which peroxisome proliferation is not induced do not show induction of liver cancer at the same doses at which $B6C3F_1$ mice do. Altered cellular metabolism leading to transient changes in cell proliferation and cell regulation is related to induction of peroxisome proliferation in rodents.

Dichloroacetic acid produces liver tumors with a different phenotype than trichloroacetic acid. Its tumorigenic effects are closely associated with differential effects on cell replication rates in tumors, normal hepatocytes, and suppression of apoptosis. There is sufficient weight of evidence to conclude that the mode of action of dichloroacetic acid at high doses in rodents includes hepatomegaly and marked cytomegaly, which are closely associated with its activity as a differential promoter with effects on increased cell replication rates in tumors and normal hepatocytes and suppression of apoptosis. High-dose treatments alter activities of key enzymes in metabolism and cell growth. Dichloroacetic acid induces liver tumorigenesis in both mice and rats by this mode of action. However, dichloroacetic acid is a minor metabolite of trichloroethylene and whether it is formed in humans has not been clearly established.

The mode of action of chloral hydrate as a weak rodent liver carcinogen is dominated by induction of peroxisome proliferation activity in male $B6C3F_1$ mice. Female mice and rats are resistant to the carcinogenic effects of chloral hydrate. Because the metabolites of chloral hydrate are trichloroacetic acid and dichloroacetic acid, the contribution to liver tumor induction of the specific modes of action of each of these metabolites is also likely; however, an overall lack of potency for chloral hydrate in the carcinogenic response is notable.

Induction of peroxisome proliferation in human liver is not a prominent feature; therefore, this key event related to trichloroacetic acid liver carcinogenesis is not likely to occur in humans. The promotional activity of dichloroacetic acid includes a significant effect on cellular metabolism, differentiation function, and proliferation that encompass a mitogenic mode of action. Repeated exposure to dichloroacetic acid results in an inhibition of both mitosis and apoptosis and eventual formation of focal eosinophilic hyperplastic lesions. Assuming that the underlying mode of action for dichloroacetic acid as a liver carcinogen in rodents is promotional events affecting and culminating in mitogenesis, genotypic species differences between mice (one transforming growth factor type-β growth factor receptor allele) and humans with two functional copies of the gene suggest that humans would be phenotypically much less susceptible to liver carcinogenesis from agents that demonstrate a mitogenic mode of action (Andersen et al. 1995).

The weak carcinogenic activity of chloral hydrate in the liver of male $B6C3F_1$ mice (with no liver cancer induction in female mice and rats) combined with lower rates of oxidation and higher rates of conjugation in humans compared with mice indicates that the mode of action in mice is not likely to be relevant to humans.

Exposure to trichloroethylene at concentrations relevant to the general public is not likely to induce liver cancer in humans. However, it is possible that much higher exposures to trichloroethylene, such as in certain high-risk occupations or in heavily contaminated locales, could result in increased risks of liver toxicity and cancer. In addition, the existence of sensitive populations due to genetics, disease, or life stage cannot be discounted.

Recommendations:

- Additional laboratory studies are needed to establish the significance of increased bile acids in relation to the hepatotoxic potential of trichloroethylene, as well as in relation to other systemic effects. Such studies will help clarify whether elevation of serum bile acids is an early indicator of changes in liver function or is a marker of exposure to trichloroethylene (or other halogenated solvents which induce this effect).
- More research is also needed to assess whether increases in serum bile acids in humans exposed to trichloroethylene are independent of discrete pathologic changes in the liver. Because histopathologic assessment would be difficult to perform in human subjects, new, highly sensitive, and noninvasive toxicologic parameters are needed to clarify the toxicologic importance of these effects of trichloroethylene.
- Additional studies of the effects of trichloroethylene on glycogen accumulation, perhaps using cultured human hepatocytes, should shed some light on the significance of this effect and its relevance to humans.
- More research is needed to determine whether an autoimmune response might play a role in trichloroethylene-mediated liver disease. Adducts formed between metabolites of trichloroethylene and liver proteins can result in the formation of neoantigens. These neoantigens can lead to antibody-dependent hepatocellular injury. The same process has been reported with chemicals such as halothane, which is well known to produce immune-mediated hepatotoxicity. Studies similar to those carried out with halothane could be instrumental in elucidating whether autoimmunity is a causal factor in the hepatotoxicity of trichloroethylene.
- Studies are needed to determine the metabolic pathway and yield for forming dichloroacetic acid from trichloroethylene either via trichloroacetic acid or via other pathway(s). If dichloroacetic acid is found to be a metabolite of concern, additional studies may be needed to understand its role in the toxicities associated with trichloroethylene.

The epidemiologic evidence for an association between liver cancer and trichloroethylene exposure is inconclusive. Excess liver cancer incidence was observed in most of the cohort studies that examined this outcome. However, cohort studies of mortality and population-based case-control studies yielded mixed results. Of particular interest is a recent case-control study that found an association between liver cancer mortality and trichloroethylene concentrations in well water in a community that was downstream from a Taiwanese factory. Although this study suffers from several methodologic weaknesses, it is the first to show an association between environmental exposures to trichloroethylene and liver cancer mortality.

5

Reproductive and Developmental Toxicity

This chapter discusses essential scientific issues about the reproductive and developmental toxicity of trichloroethylene, focusing on the issues of hazard characterization and mode of action for trichloroethylene toxicity. The chapter assesses the available information from animal, in vitro, and human studies. First, evidence for reproductive toxicity from laboratory studies is discussed. Second, developmental toxicity studies of trichloroethylene in different species are discussed, followed by information from in vitro studies that are relevant to assessing the mode of action for certain effects. Third, the evidence is considered in humans for reproductive and developmental effects together, as many epidemiologic studies evaluated reproductive and development outcomes together.

ANIMAL STUDIES OF REPRODUCTIVE TOXICITY

Studies of trichloroethylene on male reproductive end points have been done primarily in rodents. Zenick et al. (1984) reported that trichloroethylene at an oral dose of 1,000 mg/kg/day (5 days/week for 6 weeks) inhibited copulatory behavior in male Sprague-Dawley rats. Because the effects occurred during the first few weeks of exposure and returned to normal after 5 weeks, the narcotic properties of trichloroethylene were suspected to be responsible for the initial changes in copulatory behavior. No effects were observed on semen plug weights or on sperm counts, motility, or morphology (Zenick et al. 1984).

A study of male mice exposed to trichloroethylene via inhalation found significantly increased percentages of abnormal sperm at the highest test concentration (approximately150 parts per million [ppm]). Because the mice were exposed during early spermatogenesis and not for a full spermatogenic cycle, the authors concluded that the observed spermatotoxicity occurred during the first or second meiosis or during sperm maturation (spermiogenesis) (Land et al. 1981).

Forkert et al. (2002) used an inhibitory CYP2E1 monoclonal antibody to demonstrate that the enzyme CYP2E1 is involved in trichloroethylene metabolism to chloral in both the testes and epididymides. The extent of chloral formation was higher in the epididymis than in the testis and correlated with the relative levels of CYP2E1 activity present in individual tissues. Exposed

mice exhibited damage to the epididymal epithelium, which plays a central role in sperm development and functional maturity during sperm transit from the testis to the cauda epithelium. Because CYP2E1 is localized in the male reproductive tract of mice, monkeys, and humans and trichloroethylene and its P-450-derived metabolites are found in the seminal fluid of exposed humans and in the epididymides of exposed mice (Forkert et al. 2003), the effects on sperm development and functional maturity in rodents are likely to be predictive of outcomes in humans.

DuTeaux et al. (2002) proposed that the epididymis might be toxicologically analogous to the kidney. They found that enzymes involved in nephrotoxic responses (e.g., CYP2E1, soluble epoxide hydrolase, and the cysteine conjugate β-lyase) are present in the epididymis and efferent ducts of rats. The enzymes were present at higher concentrations in the efferent ducts than in the epididymis, suggesting that the proximal excurrent ducts are a potential target for compounds and metabolites that are nephrotoxicants, such as trichloroethylene.

Kumar et al. (2000a,b) also found significant decreases in total epididymal sperm count, motility, and specific activities of the steroidogenic enzymes glucose-6-phosphate dehydrogenase and 17β-hydroxysteroid dehydrogenase, with concomitant decreases in sperm testosterone, in male Wistar rats exposed by inhalation to trichloroethylene at 376 ppm (4 hours/day, 5 days/week, for 12 or 24 weeks). Fertility was reportedly reduced when the males mated with unexposed females (Kumar et al. 2000b). Follow-up investigations of whether testicular steroid precursors (cholesterol and ascorbic acid) or testosterone plays a role in trichloroethylene-induced effects showed that total cholesterol content was greater in the testes of rats exposed to trichloroethylene than in controls (Kumar et al. 2000b). The authors concluded that the findings indicated possible impairment of testicular testosterone biosynthesis, which might explain, at least in part, the reproductive inefficiency initially reported.

Another study by Kumar et al. (2001b) explored the histomorphology of the testes, sperm count and motility, and marker testicular enzymes involved in sperm maturation and spermatogenesis. They found significant reductions in body and testis weights, total cauda epididymal sperm count, and percent motile sperm after 12 and 24 weeks of exposure. Histologically, at 12 weeks, the testes exhibited fewer spermatogenic cells and spermatids in the seminiferous tubules, with some of the spermatogenic cells appearing necrotic. After 24 weeks, the testes were atrophied and harder, with smaller seminiferous tubules. Leydig cells were hyperplastic. All the tubules had Sertoli cells but were almost devoid of spermatocytes and spermatids. "Fibrines" were present in the tubular lumens. Testicular dehydrogenase and glucose-6-phosphate dehydrogenase were significantly reduced, and glutamyltransferase and β-glucuronidase were significantly increased.

The authors concluded that postmeiotic stages of spermatogenesis in rats were susceptible to trichloroethylene-induced insult. They suggested that exposure to trichloroethylene "may cause testicular toxicity, which in turn affects postmeiotic cells of spermatogenesis, Sertoli cells, and Leydig cell functions" (Kumar et al. 2001b). Whether the reproductive changes were transient or permanent was not assessed, nor were epididymal sperm counts or motility reported. It is also not clear whether the three studies reported are three separate studies, as the same design and the same trichloroethylene concentrations were used.

Xu et al. (2004) evaluated the effects of trichloroethylene on CD-1 male mice exposed by inhalation to trichloroethylene at 1,000 ppm for 6 hours/day, 5 days/week, for 1-6 weeks. There were no effects on body, testis, or epididymal weights nor on the number or percent motility of epididymal plus vas deferens sperm. More than 95% of sperm retrieved from trichloroethylene-

exposed and control mice exhibited normal morphology. In vitro incubation of sperm from trichloroethylene-exposed males with eggs from superovulated unexposed females resulted in significant decreases in the number of sperm bound per egg when the males were exposed for 2 and 6 weeks (but not after exposure of 1 or 4 weeks). In vivo fertilization with exposed males and superovulated females resulted in significant reductions in the percentage of eggs fertilized after 2 and 6 weeks of exposure to trichloroethylene (the slight reduction after 4 weeks was not statistically significant). The sperm-egg binding assay (with sperm exposed in vitro) indicated significant decreases in the number of sperm per egg when sperm were pretreated with chloral hydrate (0.1-10 μg/mL) and decreased, but to a lesser extent, with trichloroethanol treatment (0.1-10 μg/mL). The authors concluded that exposure to trichloroethylene leads to impairment of sperm's fertilizing ability, which may be attributed to the trichloroethylene metabolites chloral hydrate and trichloroethanol (Xu et al. 2004).

DuTeaux et al. (2003, 2004) investigated the bioactivation of trichloroethylene and adduct formation in the rat epididymis and efferent ducts and whether trichloroethylene caused oxidative damage to sperm. They reported that the cysteine conjugate β-lyase, which bioactivates the trichloroethylene metabolite dichlorovinyl cysteine to a reactive intermediate, was localized in the efferent ducts and epididymis (the soluble but not the mitochondrial form). Both forms of β-lyase were detected in the kidney. When rats were dosed with dichlorovinyl cysteine, no protein adducts were present in the epididymis or efferent ducts, but adducts were detected in the renal proximal tubules. Trichloroethylene can also be metabolized (and form protein adducts) through the cytochrome P-450-mediated pathway. Immunoreactive CYP2E1 was localized to the efferent ducts and corpus epididymal epithelia. Trichloroethylene metabolism was inhibited 77% when efferent duct microsomes were preincubated with an antibody to CYP2E1. Dichloroacetyl adducts were detected in epididymal and efferent duct microsomes exposed to trichloroethylene in vitro. The authors suggested that CYP2E1-dependent metabolism of trichloroethylene to reactive metabolites and the subsequent covalent binding to cellular proteins may be involved in the male reproductive toxicity of trichloroethylene (DuTeaux et al. 2003).

DuTeaux et al. (2004) conducted an in vivo study in male Sprague-Dawley rats exposed to trichloroethylene in drinking water at 0.2% or 0.4% (v/v) for 14 days. There were no treatment-related changes in testes and epididymides weight, sperm concentration, or sperm motility. Flow cytometry indicated no treatment-related differences in sperm mitochondrial potential or acrosomal stability. Trichloroethylene caused slight histologic changes in efferent ductal epithelium, coinciding with ductal localization of CYP2E1. There were no alterations in the testis or in any segment of the epididymis, but the rats exhibited significant dose-related reductions in percent fertilized ova from untreated females in vitro. However, the magnitude of the latter effect differed in rats from different sources, with rats from Charles River Laboratories having a greater percentage of fertilized oocytes than rats from a breeding colony at the University of California at Davis. Because there were no changes in sperm indices and no pathologic lesions to explain the reduced fertility, the authors used immunochemical techniques to detect oxidized sperm protein. The tests showed "halos" of oxidized proteins around the sperm head and midpiece from trichloroethylene-treated males. Dose-dependent increases in lipid peroxidation were observed in sperm from trichloroethylene-treated males as well. The authors suggested that oxidative damage to sperm may explain the reduced fertilizing capacity in trichloroethylene-exposed males and "provide another mechanism by which [trichloroethylene] can adversely affect reproductive capabilities in the male" (DuTeaux et al. 2004).

Veeramachaneni et al. (2001) reported that a mixture of drinking water pollutants, including trichloroethylene, caused alterations in mating desire and ability, sperm quality, and Leydig cell function in rabbits. Although there is no way to parse out the contribution, if any, from trichloroethylene on these rather subtle and subjectively assessed effects, this is the only paper on trichloroethylene that used the rabbit, which is considered the most sensitive species for detection of male reproductive toxicity.

In a study of B6D2F_1 pregnant mice, no effect of trichloroethylene was observed on maternal, reproductive, or offspring parameters at an oral dose of 140 mg/kg/day during gestation (Cosby and Dukelow 1992). The authors also performed in vitro studies of mouse eggs cultured with cauda epididymal sperm and trichloroethylene or its metabolites—dichloroacetic acid, trichloroacetic acid, and trichloroethanol—to assess the effects on fertilization. No effect on fertilization was found with trichloroethylene at concentrations up to 1,000 ppm. Dichloroacetic acid and trichloroacetic acid each showed a dose-related decrease in the percent of eggs fertilized that was significant at 1,000 and 100 mg/kg, respectively. Trichloroacetic acid and trichloroethanol individually and in combination also exhibited significant reductions in the percentage of fertilized embryos, but the combination did not exhibit a synergistic effect (Cosby and Dukelow 1992).

NTP conducted reproductive-assessment-by-continuous-breeding studies of trichloroethylene in CD-1 mice (NTP 1986a) and Fischer 344 rats (NTP 1986b). Both species received feed containing microencapsulated trichloroethylene at 0.15%, 0.30%, and 0.60% (w/w). In mice, the most significant finding was perinatal mortality (61% in the high-dose group versus 28% in the controls). Sperm motility was reduced by approximately 45% in F_0 males and by 18% in F_1 males. No effects on mating, fertility, or reproductive performance were found (NTP 1986a).

In the Fischer 344 rats, a monotonic trend was present for fewer litters per mating pair (from 3.5 in controls to 2 in the high-dose group; the middle- and high-dose groups had 9% and 16% fewer pups per litter, respectively). Crossover mating indicated reduced mating (75%) in groups with a treated parent. Number of pups per litter, viability, and weight of the pups were not affected. There were no changes in sperm indices. Reduced male body weights, reduced absolute testis weights, and increased adjusted seminal vesicle weights were found at necropsy. The conclusion was that trichloroethylene produced some general toxicity (reduced body weight gains, and increased relative liver and kidney weights) at all doses, whereas reduced reproductive indices were observed only in the F_1 rats at the middle and high doses (NTP 1986b).

Manson et al. (1984) investigated reproductive performance in female Long-Evans hooded rats exposed to trichloroethylene by gavage at doses of 10, 100, or 1,000 mg/kg/day (in corn oil). The rats were dosed two weeks prior to mating, during the one-week mating period (5 days per week), and from gestational day 0 to 21 (7 days per week). Trichloroethylene and its major metabolites, trichloroacetic acid and trichloroethanol, were measured in the female reproductive organs and neonatal tissues. At the end of the premating period, trichloroethylene concentrations were uniformly high in fat, adrenal glands, and ovaries across groups, while uterine tissue had relatively high concentrations of trichloroacetic acid. Female fertility was unaffected. Four of 23 females treated with trichloroethylene at 1,000 mg/kg/day died (and one had a fully absorbed litter), and weight gain was significantly reduced throughout the treatment period. Neonatal survival was significantly reduced at 1,000 mg/kg/day, with the majority of deaths among female offspring at the time of birth. Trichloroacetic acid concentrations in blood, liver, and milk contents of the stomach of female (but not male) neonates exhibited a dose-

related increase across groups. The authors concluded that oral exposure to trichloroethylene at doses below those that cause maternal toxicity did not affect fertility or pregnancy outcome and that the accumulation of trichloroethylene and trichloroacetic acid in ovaries, adrenal glands, and uteri had no impact on mating success. No information on any structural anomalies was provided. The lack of any effects on offspring survival argues against any functional consequences, except for the preferential loss of offspring females at birth, which remains unexplained.

Berger and Horner (2003) exposed female rats to a number of male reproductive toxicants, including trichloroethylene and tetrachloroethylene, and assessed the fertilization of their oocytes by sperm from unexposed males in vitro. Female Simonson albino rats were administered drinking water containing trichloroethylene at 0.45% for 2 weeks. They were induced to ovulate, and their oocytes were incubated with semen from unexposed male rats from the same colony. Trichloroethylene significantly reduced fertilizability of the oocytes (46% versus 57% in the vehicle control females [$P < 0.005$]). Trichloroethylene also significantly reduced the number of penetrated sperm per oocyte (0.70 per oocyte versus 0.81 per oocyte in the vehicle control [$P < 0.05$]). Oocytes from trichloroethylene-exposed females also had reduced ability to bind sperm plasma membrane proteins compared with oocytes from the vehicle controls ($P < 0.05$).

Tetrachloroethylene, administered in drinking water at 0.9%, reduced the percentage of females ovulating compared with the vehicle values (53% versus 78%, $P < 0.05$). There were no effects on oocyte fertilizability or on the number of penetrated sperm per oocyte. Nose-only inhalation exposure of females to tetrachloroethylene at 1,700 ppm for two 1-hour periods per day for 2 weeks slightly reduced the fertilizability of oocytes (from 85% to 80%, $P < 0.05$). The number of penetrated sperm per oocyte was more obviously reduced (1.6 for exposed females versus 2.5 for unexposed females). There were no clinical signs of toxicity. Berger and Horner (2003) view their work as the first documented in vivo effect on oocyte fertilizability by a reproductive toxicant for a female mammal (for trichloroethylene and tetrachloroethylene). Because there is evidence that both compounds are male reproductive toxicants via a mechanism that does not involve the endocrine system, these results in females support their hypothesis.

ANIMAL STUDIES OF DEVELOPMENTAL TOXICITY

Avian and Mammalian Species

The avian explant model is used for descriptions and mechanistic studies of heart development and teratogenesis (as well as for other organ system development) because of the conservation of developmental stages and perturbations across vertebrates, especially birds and mammals, and because of the access to, and visibility of, developing avian structures in ovo and in vitro. Studies of trichloroethylene in the in ovo development of chicks have reported increased mortality and developmental defects, including lighter pigmentation, edema, evisceration (failure of abdominal wall closure, gastroschisis), decreased growth, beak malformations, club foot, and patchy feathering (Bross et al. 1983). Loeber et al. (1988) reported cardiac defects that involved inflow and outflow abnormalities, including septal defects, conotruncal abnormalities, atrioventricular canal defects, hypoplastic ventricle, and abnormalities in cardiac muscle.

Dorfmueller et al. (1979) compared the effects of timing of exposure to trichloroethylene on reproductive outcomes of female Long-Evans hooded rats exposed to trichloroethylene by inhalation at concentrations of 1,800 ± 200 ppm. Groups of rats were exposed before mating only, during pregnancy only, and throughout pre-mating, mating, and pregnancy. There were no effects of any exposure paradigm on maternal body or liver weights or on pre- or post-implantation loss, litter size (live, dead, resorbed, total), resorption rate, fetal body weight, or sex ratio. Fetal skeletal anomalies (predominantly incomplete ossification of sternum) and soft tissue anomalies (displaced right ovary) were significantly increased only in the group exposed during gestation. The investigators considered these effects to be evidence of developmental delay in maturation rather than teratogenesis. Variable effects were observed in the mixed function oxidase enzyme assay which did not correlate with treatment or pregnancy state. However, when the two groups with and the two groups without gestational exposure were compared, a significant increase in ethoxycoumarin dealkylase was associated with gestational exposure. Behavioral evaluation of the pups indicated no effect of treatment in general motor activity in any groups at any age. A reduction in postnatal body weights was observed in the offspring of mothers with pregestational exposure. The authors concluded that "No results indicative of treatment-related maternal toxicity, embryotoxicity, serum teratogenicity, or significant behavioral deficits were observed in any of the treatments groups" (Dorfmueller et al. 1979, p. 153).

Schwetz et al. (1975) exposed timed-pregnant Sprague-Dawley rats and Swiss Webster mice to trichloroethylene by inhalation at a concentration of 300 ppm (twice the maximum allowable excursion limit for human industrial exposure defined by the American Conference of Governmental Industrial Hygienists) for 7 hours/day on gestational days 6-15. No effects from trichloroethylene were found in rat or mouse dams (except for a statistically significant 4-5% reduction in maternal body weights in rats) or conceptuses using standard Segment II developmental toxicity assessments, including pre- and post-implantation loss, litter size, fetal body weight, crown-rump length, and external, visceral, skeletal, and total malformations and variations.

Smith et al. (1989, 1992) studied the trichloroethylene metabolites trichloroacetic acid and dichloroacetic acid in pregnant Long-Evans rats and found that both metabolites reduced body weight and growth and produced cardiac defects. The most common findings after treatment with trichloroacetic acid were levocardia (at 330 mg/kg/day and greater) and interventricular septal defect (800 mg/kg/day and greater). With dichloroacetic acid, resorptions significantly increased at 900 mg/kg/day; the most common cardiac malformations were a defect between the ascending aorta and right ventricle (at 140 mg/kg/day and greater), levocardia (at 900 mg/kg/day and greater), and intraventricular septal defect (at 1,400 mg/kg/day and greater). Thus, trichloroacetic acid appears to be more potent than dichloroacetic acid in causing cardiac teratogenicity, although both compounds exhibited dose-response relationships. The authors did not find a no-observed-adverse-effect level for trichloroacetic acid, but they concluded that the no-observed-adverse-effect level for the developmental toxicity of dichloroacetic acid in rats was 14 mg/kg/day (Smith et al. 1992).

A follow-up series of four studies on dichloroacetic acid were performed to determine the most sensitive period of development and to further characterize the heart defects (Epstein et al. 1992). The heart defects found were predominantly high interventricular septal defects and, less commonly, interventricular septal defects. The authors suggested that high interventricular septal defects are a specific type of defect produced by a failure of proliferating interventricular

septal tissue to fuse with the right tubercle of the atrioventricular cushion tissue. In the proposed model, of the three foramina (primum, secundum, and tertium) initially present, a single interventricular foramen is eventually obliterated. They also proposed that dichloroacetic acid interferes with closure of the interventricular foramen tertium, allowing the aorta to retain its embryonic connection to the right ventricle. In these studies, disruption of these septation processes did not affect the aortic connection with the left ventricle. The authors rightly questioned why dichloroacetic acid has a selective effect on fetal cardiogenesis. They speculated that perhaps the dichloroacetic acid target is a unique cell type at a unique time—the biochemical differentiation of cardiocytes (Epstein et al. 1992).

Dawson et al. (1990) reported that continuous delivery of trichloroethylene into the gravid uteri of Sprague-Dawley rats resulted in increased incidence of fetal heart malformations (on a fetal basis—that is, number of fetuses with heart defect[s]/number of fetuses examined). The incidence was 9% with trichloroethylene at 15 ppm and 14% with trichloroethylene at 1,500 ppm, compared with a 3% incidence in the control group (an approximately 36% increased incidence at a 100-fold increase in exposure).

In another study with a more conventional experimental design, Dawson et al. (1993) exposed Sprague-Dawley rats to trichloroethylene (1.5 or 1,100 ppm) or dichloroethylene (0.15 or 110 ppm) in drinking water before pregnancy, during pregnancy, and both before and during pregnancy. They found no differences among groups in the percentage of live births, uterine implants, or resorptions. There were also no differences among groups in congenital abnormalities other than cardiac defects. However, it is unclear how completely teratogenesis was evaluated. Of the 238 fetuses in the control group, 3% had cardiac defects (2.5% of the more than 600 fetuses in control groups in this and previous studies exhibited cardiac defects). In this study, the high concentrations of trichloroethylene and dichloroethylene were 733% higher than the low concentrations, but the increased incidence of cardiac malformations was only 13% with trichloroethylene and 12% with dichloroethylene. The dose-response curve was extraordinarily flat.

To evaluate the proximate teratogen(s) responsible for the fetal cardiac malformations associated with trichloroethylene and dichloroethylene, Johnson et al. (1998a,b) tested several metabolites of the two compounds in Sprague-Dawley rats. They found an increased incidence of cardiac malformations with trichloroacetic acid at a concentration of 2,730 ppm (10.53% versus 2.15% in the cumulative control group; $P = 0.0001$ for fetuses and $P = 0.0004$ for affected litters). The cardiac malformations included atrial septal defect, perimembranous ventricular septal defect, pulmonary artery hypoplasia, aortic hypoplasia, mitral valve defect, muscular ventricular septal defect, and pulmonary valve defect. Increased cardiac defects were not found with the other metabolites tested (monochloroacetic acid, trichloroethanol, trichloroacetaldehyde, dichloroacetaldehyde, carboxymethylcysteine, and dichlorovinyl cysteine). The metabolite dichloroacetic acid was not evaluated in this study. The investigators asserted that the low number of cardiac defects found in the metabolite groups (other than trichloroacetic acid) does not preclude teratogenicity, because the study might not have had enough statistical power to detect an effect. They also asserted that the study does not prove that trichloroacetic acid is a human cardiac teratogen. Limitations associated with the study include discrepancies in the number of affected hearts and fetuses reported in the paper and failure to disclose that the control group was not concurrent.

Johnson et al. (2003) sought to identify a threshold dose of trichloroethylene in rats. They reclassified the data reported by Dawson et al. (1993) and assessed them with information

on two lower test concentrations (0.0025 and 0.25 ppm). The authors concluded that their analysis identified "a threshold level of less than [0.25 ppm trichloroethylene] above which rats exposed to increasing levels of [trichloroethylene] during pregnancy have increasing incidences of cardiac malformations in their fetuses."

Fisher et al. (2001) also evaluated trichloroethylene (500 mg/kg/day) and the metabolites trichloroacetic acid (300 mg/kg/day) and dichloroacetic acid (300 mg/kg/day) for teratogenicity. The two metabolites produced significantly reduced fetal body weights on both a per fetus and a litter basis. They found no statistically significant increases in the incidence of fetal heart malformations by litter or fetus for trichloroethylene or the two metabolites. The incidences were 4.5%, 3.3%, and 4.7% for trichloroethylene, trichloroacetic acid, and dichloroacetic acid, respectively. Interestingly, the rate of cardiac malformations observed in the treatment groups, although not different from the concurrent controls, was similar to those reported in the treatment groups by Johnson et al. (1998a,b) and Dawson et al. (1993). Of note, the frequency of the abnormalities in the soybean oil control group were higher in this study (6.5%) than in the control groups (individually and grouped) of Johnson et al. (1998a,b, 2003) and Dawson et al. (1993). Such a difference would decrease the power to detect a difference.

Collier et al. (2003) studied the effects of trichloroethylene, dichloroethylene, and trichloroacetic acid on gene expression in rats during cardiac development. They found up-regulated transcripts including genes associated with stress response (*Hsp70*) and homeostasis (several ribosomal proteins). Down-regulated transcripts included extracellular matrix compounds (GPI-p137 and vimentin) and Ca^{2+} responsive proteins (Serca-2 Ca^{2+}-ATPase and β-catenin). Down-regulated sequences appear to be associated with cellular housekeeping, cell adhesion, and developmental processes. Two possible markers for fetal trichloroethylene exposure were Serca-2 Ca^{2+}-ATPase and GPI-p137.

Collier et al. (2003) considered that cardiac insufficiency is a "plausible explanation" for the reduced incidence of reported malformations from in utero exposure to trichloroethylene. They argued that a lack of exposure studies in rats and mice terminated before embryonic day 18 (mice) or day 21 (rats) would exclude findings of gross cardiac defects inconsistent with life that could be identified only early in gestation (these conceptuses would die before term). The authors therefore associated "the limited reports of cardiac defects associated with [trichloroethylene] exposure with timing of the analysis, not an absence of cardiac-related effects from exposure" (Collier et al. 2003, p. 495). However, the timing of necropsy and fetal heart examinations in rodent models has been the same for researchers reporting rodent fetal heart malformations and researchers not reporting those effects. Consistent with the changes in gene regulation that Collier et al. observed, trichloroethylene and trichloroacetic acid, but not trichloroethanol or chloral, inhibited in vitro gap-junction-mediated intercellular communication, an important part of cellular adhesion and cardiac development (Klaunig et al. 1989).

Coberly et al. (1992) used the mouse embryo chimera assay to evaluate the effects of trichloroethylene on preimplantation embryos. Superovulated female CD-1 (Swiss) mice were treated with trichloroethylene intraperitoneally (0, 0.01, 0.02, or 10 μg/kg) or by gavage (0, 0.1, and 1.0 μg/kg; 0, 48.3, and 483 mg/kg) when the embryos were traversing the pronuclei stages of development. Embryos were flushed from excised oviducts and scored for numbers, embryonic stages, and viability for each female. The stages included degenerate and 1-cell embryos, and 2- and 4-cell embryos. All 4-cell embryos from females within a dose group were pooled, and the chimeras constructed from them. No treatment-related effects were seen on the total number of

embryos recovered from the oviducts of trichloroethylene-treated females, and no significant cell proliferation decreases were observed for any of the experimental chimeric embryos.

Other Species

Relevant toxicity studies have been performed in animal models other than rodent and avian species, including daphnids and amphibians. Niederlehner et al. (1998) evaluated the reproductive response of the daphnid *Ceriodaphnia dubia* to industrial chemicals alone and as mixtures of trichloroethylene, benzene, toluene, ethylbenzene, *m*-xylene, and tetrachloroethylene. The reproductive median inhibition concentration was 82 μM for trichloroethylene and 4 μM for tetrachloroethylene. Mixtures of trichloroethylene, benzene, and toluene had effects at concentrations below their individual lowest-observed-effect levels. In addition, observed responses to mixtures differed significantly from that predicted from a concentration-addition model, with the predicted relationship overestimating mixture toxicity (Niederlehner et al. 1998).

The Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) has been used to study the developmental toxicity of trichloroethylene. Trichloroethylene has tested positive in this assay (Fort et al. 1991, 1993). The trichloroethylene metabolites dichloroacetic acid, trichloroacetic acid, trichloroethanol, and oxalic acid have also tested positive in the FETAX assay, but each was significantly less toxic than trichloroethylene. It was suggested that trichloroethylene oxide, a highly embryotoxic epoxide intermediate formed from mixed-function oxidation-mediated metabolism, might play a significant role in the developmental toxicity of trichloroethylene (Fort et al. 1993).

Embryonic larvae of four North American amphibian species—wood frogs (*Rana sylvatica*), green frogs (*Rana clamitans*), American toads (*Bufo americanus*), and spotted salamanders (*Ambystoma maculatron*)—were exposed to tetrachloroethylene and its metabolites, trichloroethylene and *cis*- and *trans*-dichloroethylene. Tetrachloroethylene and trichloroethylene were teratogenic to amphibian embryos, with median effective concentrations (EC_{50}s) (malformations) of 12 mg/L for tetrachloroethylene in wood frogs and 40 mg/L for trichloroethylene in green frogs; these concentrations did not affect embryonic survival. American toads were less sensitive, with no EC_{50} for developmental abnormalities attained at the highest test concentrations (tetrachloroethylene at 45 mg/L and trichloroethylene at 85 mg/L) (McDaniel et al. 2004).

In Vitro Studies

Saillenfait et al. (1995) used rat whole embryo cultures to retain embryonic structural integrity and to preclude the presence of maternal absorption, distribution, metabolism, and excretion. They exposed explanted Sprague-Dawley embryos (gestational day 10) to trichloroethylene, tetrachloroethylene (with or without microsomes), or one of four chlorinated compounds (trichloroacetic acid, dichloroacetic acid, chloral hydrate, and trichloroacetyl chloride). They found concentration-dependent decreases in growth and differentiation indices and increases in the incidence of morphologically abnormal embryos with all the test chemicals. Trichloroethylene and tetrachloroethylene produced qualitatively similar patterns of

abnormalities, whereas their metabolites produced distinguishable dysmorphic profiles. The presence of hepatic microsomal fractions in the culture medium enhanced embryotoxic effects. Embryo lethality was defined as loss of heartbeat; the percentage of explants with a heartbeat ranged from 36% with trichloroethylene and 43% with tetrachloroethylene to 86% and 100%, respectively, in the presence of the microsomal biotransformation system. Heart defects were not mentioned. All treatments at higher doses caused a treatment-related reduction in the first branchial arch, and an abnormal brain was the most prominent effect noted. Incomplete closure of the neural tube was also noted. Chloral hydrate caused pericardial dilation (at 2 mM, with 100% embryo lethality at 2.5 mM). With respect to embryo lethality, the order of potency for metabolites was chloral, trichloroacetyl chloride, dichloroacetic acid. The dose-response curve for embryo lethality was steep. Trichloroethylene at 15 mM caused malformations but no embryo deaths, but 30 mM was 90% embryo lethal. Tetrachloroethylene caused 10% embryo lethality at 7.5 mM and 83.5% embryo lethality at 15 mM.

A number of in vitro studies of the effects of trichloroethylene and its metabolites on cardiac valve formation have been performed. The basic events of cardiac valve formation in mammals (including humans and laboratory animals) and birds are as follows:

1. Early in development (in utero or in ovo), the heart is a hollow, linear, tube-like structure with two cell layers. The outer surface is a myocardial cell layer, and the inner luminal surface is an endothelial cell layer. Between the two cell layers is extracellular matrix.
2. At a specific time in development, a subpopulation of endothelial cells lining the atrioventricular canal detaches from adjacent cells and invades the underlying extracellular matrix (Markwald et al. 1984). This event is termed an epithelial-mesenchymal cell transformation, when at least three distinct events occur: endothelial cell activation (chick stage 14), mesenchymal cell formation (chick stage 16), and mesenchymal cell invasion (migration) into the extracellular matrix (chick stages 17 and 18) (Boyer et al. 2000a).
3. Endothelial-derived mesenchymal cells migrate toward the surrounding myocardium and begin proliferating to populate the entire atrioventricular canal extracellular matrix.
4. The cardiac mesenchyme provides the cellular constituents for the septum intermedium and the valvular leaflets of the mitral (bicuspid) and tricuspid atrioventricular valves. The septum intermedium subsequently contributes to the lower portion of the interatrial septum and the membranous portion of the interventricular septum (Markwald et al. 1984, 1996; Boyer et al. 2000).

The chick stage 16 atrioventricular canal can be removed from the embryo and cultured in vitro on a three-dimensional hydrated collagen gel. During the 24 to 48 hours of incubation, all the stages described above occur in vitro and can be studied with or without test chemical exposures (e.g., Mjaatvedt et al. 1987, 1991; Loeber and Runyan 1990; Ramsdell and Markwald 1997). The in vitro model has identified a number of molecules as being involved with this transformation (e.g., fibronectin, laminin, galactosyltransferase [Mjaatvedt et al. 1997]; components of the extracellular matrix [Mjaatvedt et al. 1991]; and smooth muscle α-actin and transforming growth factor β3 [Nakajima et al. 1997; Ramsdell and Markwald 1997]).

Because trichloroethylene was implicated in heart defects of the chick (Bross et al. 1983), Boyer et al. (2000) explanted chick stage 16 atrioventricular canals onto gels with medium containing trichloroethylene at 50, 100, 150, 200, or 250 ppm. The explants were evaluated for epithelial-mesenchymal transformation, endothelial cell density, and immunohistochemistry.

Atrioventricular canal explants for chick stage 17 embryos were also cultured with no chemicals. Then, medium containing 0 or 250 ppm was added for 30 minutes, and the cell migration assay was performed. Trichloroethylene affected several elements of the epithelial-mesenchymal cell transformation process, including blockage of the endothelial cell-cell separation process that is associated with endothelial activation, inhibition of mesenchymal cell formation in a dose-dependent pattern, and no effect on the cell migration rate of the fully formed mesenchymal cells. The expression of three proteins selected as molecular markers of the epithelial-mesenchymal transformation was analyzed. Trichloroethylene inhibited the expression of transcription factor Mox-1 and extracellular matrix protein fibrillin 2 but had no effect on expression of smooth muscle α-actin. The authors suggested that trichloroethylene might cause cardiac valvular and septal malformations by inhibiting early endothelial separation and early events of mesenchymal cell formation in the embryonic heart (Boyer et al. 2000). Another interpretation (Hoffman et al. 2004) is that trichloroethylene affects the adhesive properties of endocardial cells. On the other hand, other have questioned the relevance of this study based on concerns that the concentrations used would not be tolerated by animals or achieved in humans (Dugard 2000). No direct experimental data are available that address trichloroethylene concentrations present in cardiac tissue in vivo.

Hoffman et al. (2004) proposed the using a whole embryo explant culture as a better system to evaluate the effects on the formation of the valves and septa of the heart, as anatomic relationships among tissues and organs are maintained and embryonic development can be monitored over the course of the experiment. Because mesenchymal cells first appear in the atrioventricular canal extracellular matrix at chick stage 16, they explanted stage-14 embryos for culture with trichloroethylene concentrations of 0, 10, 40, or 80 ppm. Only comparably staged and morphologically identical control and trichloroethylene-treated embryos were analyzed further by scanning laser confocal microscopy to assess cellular characteristics of the endocardial cushion tissues. With a trichloroethylene concentration of 80 ppm, there was a reduction (58.3% of the control value) in total cells of the atrioventricular cushion and an altered distribution of mesenchymal cells within the cushion. (Embryos treated with 40 ppm trichloroethylene were not assessed.) The authors also tested trichloroacetic acid in their whole embryo explant systems, and it too altered the distribution of cells in the endocardial cushions (Hoffman et al. 2004).

Using an in vitro mouse conceptus model in which haloacetic acids were added individually to culture medium, Hunter et al. (1996) showed that haloacetic acids generally are capable of causing altered development of the neural tube, eye and pharyngeal arches, and heart. With the exception of higher (≥250 μM) concentrations of monochloracetic acid, no increased embryo death was seen. Trichloroacetic acid was not teratogenic at 1,000 μM. At 2,000 μM, increased neural tube defects and fewer somites were observed. At 3,000 μM, an increase in eye, pharyngeal arch, and heart defects was seen. The cardiac anomalies were predominantly incomplete looping; a reduction in cardiac length beyond the bulboventricular fold and a reduction in the caliber of the heart tube lumen also were observed. Dichloroacetic acid was not teratogenic at 734 μM, but a significant, albeit inconsistent, decrease in somite number was variably observed at 1,468 μM or greater. Increased neural tube defects occurred at 5,871 μM, whereas pharyngeal defects and cardiac defects were observed at concentrations of 7,339 μM or greater. Extremely high concentrations (11,010 μM) caused rotational, eye, and somite dysmorphology. Virtually all haloacetic acids produced neural tube defects, but the potency varied by four orders of magnitude. The authors calculated benchmark concentrations for neural tube defects (defined as the lower 95% confidence interval of the concentration of acid required

to produce a 5% increase in the number of embryos with neural tube defects) of 91, 1,336, and 2,452 μM for monochloroacetic acid, trichloroacetic acid, and dichloroacetic acid, respectively. Generally, the chloroacetic acids were less potent than the bromoacetic acids but more potent than the fluoroacetic acids (Richard and Hunter 1996). This evidence that multiple halogenated compounds might be teratogenic supports the need for studies of outcomes after combined exposures.

Direct extrapolation of the results of direct embryo culture studies is limited because maternal absorption, excretion, and metabolism do not occur in in vitro systems. In addition, no conceptuses were exposed simultaneously to multiple haloacetic acids, all of which are frequently low-level water disinfection products, or to other common coexposure chemicals, including compounds metabolically upstream and downstream of the haloacetic acids, such as trichloroethylene and chloral hydrate. However, such models allow intrinsic toxicity to be evaluated.

Finally, one in vitro assay used bovine coronary endothelial cells cultured in medium containing 10% fetal bovine serum with antibiotics to suggest that endothelial nitric oxide synthase might be involved in trichloroethylene-mediated toxicity. Proliferating endothelial cells were treated with trichloroethylene at 0-100 μM and then stimulated with the calcium ionophore A23187 to determine changes in endothelial cells and endothelial nitric oxide synthase, nitric oxide, and superoxide anion generation. Trichloroethylene decreased concentrations of heat shock protein associated with endothelial nitric oxide synthase by 46.7% and inhibited vascular endothelial growth-factor-stimulated endothelial cell proliferation by 12% to 35%. These data show that trichloroethylene alters heat shock protein interactions with endothelial nitric oxide synthase and induces endothelial nitric oxide synthase to shift nitric oxide to superoxide-anion generation. The findings provide new insight into how trichloroethylene alters endothelial and endothelial nitric oxide synthase function to impair vascular endothelial growth-factor-stimulated endothelial proliferation. Such changes in endothelial function play an important role in the development of heart defects (Ou et al. 2003).

HUMAN STUDIES OF REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

Currently, studies of the human reproductive and development effects of trichloroethylene consist of (1) retrospective, community-based studies of multiple pregnancy outcomes among residents of neighborhoods with varying documentation of trichloroethylene or trichloroethylene-related exposures; (2) studies of reproductive outcomes of men and women with nonquantitative occupational exposure to multiple, ill-defined organic solvents; (3) limited studies of health outcomes of children exposed to trichloroethylene, including intrauterine exposure; and (4) evaluations of spermatogenesis and sexual function among men with occupational exposure to high concentrations of trichloroethylene or trichloroethylene-related compounds. The following discussion provides a qualitative overview of the epidemiologic evidence. A more critical evaluation of relevant studies in terms of methods, exposures, and results is necessary to fully characterize the reproductive and developmental hazards of trichloroethylene (see Chapter 2 for guidance on how this should be done).

Community-Based Studies

Woburn, Massachusetts

Birth outcomes have been studied in communities of East Woburn, Massachusetts, that were served between 1964 and 1979 by wells contaminated with trichloroethylene (267 parts per billion [ppb]) and tetrachloroethylene (21 ppb). A health survey of 5,010 residents of Woburn (about 50% of the population) by Lagakos et al. (1986) found an increased likelihood of exposure to contaminated well water and ear and eye anomalies (odds ratio [OR] = 14.9; $P < 0.0001$) and perinatal deaths (OR = 10.0, $P = 0.003$) between 1970 and 1982. A combination of central nervous system, chromosomal, and oral cleft anomalies was also reported to be increased, but a review of data and the fact that this is an unconventional grouping of outcomes suggested that the finding was not plausibly related to exposure to the contaminated wells. Although no other birth defects or anomalies were reported, statistical power was limited. Spontaneous abortion and low birth weight were not increased; however, the study used a nonstandard cutoff weight to assess low birth weight (2,722 g versus 2,500 g).

A study by the Massachusetts Department of Public Health (MDPH/CDC/MHRI 1994) of the same population indicated the possibility of increased risk for small-for-gestational-age-babies in the context of exposure in the third trimester of pregnancy, particularly among teenage women (OR = 6.37; 95% confidence interval [CI] = 2.39, 16.99), and for preterm birth among older mothers with exposure in the third trimester (OR = 2.66; 95% CI = 1.14, 6.19). Others reported an interaction between maternal age and trichloroethylene (Yauck et al. 2004) and the similar compound tetrachloroethylene (Sonnenfeld et al. 2001) as well as other compounds (Fox et al. 1994; Jacobson et al. 1998). However, gestational age was not reported for more than half of the sample, making these observations unreliable.

The prevalence of structural birth defects was evaluated retrospectively between January 1975 and December 1984 and prospectively between January 1989 and March 1991. Over 4,500 hospital records were reviewed for the retrospective study, and over 11,000 for the prospective study. Ascertainment methods increased the possibility of a type II error for many birth defects, particularly congenital heart disease. The prevalence of choanal atresia (OR = 8.33, 95% CI = 2.37, 26.25; OR = 6.6, 95% CI = 1.99, 19.19) and hypospadias (OR = 1.59, 95% CI = 1.02, 2.45) was significantly higher in Woburn during the period of well contamination than in two national referent populations. Although the rates remained higher after well closure, the ascertainment methods for the post-well-closure period were more complete than during the contamination period. A referent population (such as from a retrospective analysis during the contamination years of the 12 noncontaminated communities used in the prospective study) was not included.

Camp Lejeune, North Carolina

Studies of developmental outcomes have been performed at the U.S. Marine Corps Base at Camp Lejeune, North Carolina, where drinking water was found to be contaminated with chlorinated volatile organic compounds, trichloroethylene, tetrachloroethylene, dichloroethylene, and lead. Exposure to these compounds was documented over a period of 34 months but likely occurred for years, perhaps as long as 30 years. Concentrations of trichloroethylene ranged from 8 to 1,400 ppb, dichloroethylene ranged from 12 to 407 ppb, and tetrachloroethylene ranged

from 76 to 215 ppb, depending on the water system and the time of testing. From the evaluations at Camp Lejuene to date, two potentially plausible findings appear. Trichloroethylene exposure appears to be associated with significantly smaller male infants, whether measured as a continuous variable or as a dichotomous variable (ATSDR 1998; Sonnenfeld et al. 2001). Among exposed male infants, adjusted mean birth weight was reduced by 312 g (90% CI = -540, -85; $P < 0.01$), and the prevalence of small for gestational age increased (OR = 3.9, 90% CI = 1.1, 11.9), whereas no difference was found in female infants.

Although such gender differences are not readily explained and have not been associated with trichloroethylene in other studies, male susceptibility has been seen with other chemicals, such as polychlorinated biphenyls and dioxins (Dewailly et al. 1993; Rylander et al. 1995). For tetrachloroethylene, two exposed subgroups appeared at greater risk of adverse outcomes: women over the age of 35 and those with a history of fetal loss (adjusted OR = 2.1, 90% CI = 0.9, 4.9; OR = 2.5; 90% CI = 1.5, 4.3). The adjusted differences in mean birth weight in the tetrachloroethylene-exposed infants in the two subgroups were -130 g (90% CI = -236, -23) and -104 g (90% CI = -174, -34), respectively. Increased environmental risk of birth defects among older women has been observed for trichloroethylene (Yauck et al. 2004), ethanol (Jacobson et al. 1996; Jacobson, et al. 1998), and smoking (Backe 1993; Fox et al. 1994). The association between prior fetal deaths and risk appeared to increase with the number of fetal deaths, increasing the probability that it was not a chance observation.

Limitations to the ATSDR (1998) study include the possibility of misclassification, particularly the possibility that unexposed mothers were included in the "exposed" population. This is more likely to be true in the tetrachloroethylene and "long trichloroethylene exposed" groups than in the "short trichloroethylene exposed" groups and would decrease the power to detect a difference and lead to a bias toward the null. The information about exposure for any individual is crude, as no information about water consumption was available, nor was information available about showering or other hot water activities, which would contribute to exposure by dermal and inhalation routes. Biologic monitoring information was also not available.

The clinical determination of gestational age from retrospective data is difficult and, in the ATSDR study, underestimates of gestational age likely occurred with birth weight used as a criterion because large-for-gestational-age preterm infants were removed from the study. Such an underestimate would decrease power and attenuate differences in the number of small-for-gestational-age infants between exposed and unexposed women. Removal of large-for-gestational-age preterm infants substantially decreased the number of preterm infants, which potentially decreased the power to detect a difference in prematurity rates. Data on tobacco and alcohol—other important effect modifiers—were not available. However, these exposures are less likely to have affected the exposure groups differentially.

ATSDR (2003) plans another study to assess birth defects and childhood cancer (leukemia, nonHodgkin's lymphoma) prevalence among children exposed to contaminated drinking water at Camp Lejeune. Surveys have been conducted to identify the study population and confirm the health outcomes reported by parents. A full study is planned to include all confirmed cases of birth defects and childhood cancers and an assessment of exposure to trichloroethylene and other drinking water contaminants by modeling the water system.

Santa Clara County, California

After the identification of well contamination with 1,1,1-trichloroethane, a solvent that shares some of the same principal metabolites as trichloroethylene (trichloroethanol and trichloroacetic acid), the public reported an increased number of spontaneous abortions and cases of congenital heart disease. A series of studies were done evaluating pregnancy outcomes (Deane et al. 1989; Wrensch et al. 1990a,b) and congenital heart disease (Swan et al. 1989). Deane et al. (1989) reported a higher rate of spontaneous abortions and congenital anomalies among exposed women (n = 250). The relative risk of congenital anomalies considered as a single entity was 3.1 (95 % CI = 1.1, 10.4). A later study by the same investigators (Wrensch et al. 1990a) expanded on this study and included an additional exposed area (n = 1,105). The analysis of the larger data set did not confirm the previous finding of an increase in spontaneous abortions in exposed women. An additional report (Wrensch et al. 1990b) that provided hydrogeologic assessment of the amount of exposure in two exposed census tracts found that the tract with higher concentrations of 1,1,1-trichloroethane had a lower rate of spontaneous abortions than the tract with lower 1,1,1-trichloroethane concentrations. The sample size was too small for statistical evaluation of birth defects.

The cluster of congenital heart disease in Santa Clara County was confirmed, but Swan et al. (1989) suggested that it was not likely to be related to 1,1,1-trichloroethane because the increased prevalence of congenital heart disease was not consistent across the time period when exposure occurred. However, most cases of congenital heart disease (9 of 12 cases) occurred in a region not served by the well that was the focus of the study. In fact, the cluster was closer to a well that contaminated by about 80-fold less 1,1,1-trichloroethane and smaller amounts of dichloroethylene, with perhaps slightly different time periods. The impre*cis*e assessment of exposure is such that the manuscript does not add substantial information for risk assessment.

The assessment of birth defects in the study of Wrensch et al. (1990a) included an analysis of 36 of 166 reported cases of birth defects. Only about 35% of women were interviewed for birth defect ascertainment because of out-migration. Women who remained in the area might not represent the total exposed population; those who left the area could plausibly have a higher rate of offspring with birth defects than those who remained there. A 4-fold increase in prevalence of malformations was seen in the original exposed area compared with the original unexposed area (Deane et al. 1989), but this was not replicated in the comparison of the added exposed and control areas (Wrensch et al. 1990a). In addition, the confidence intervals for the association between birth defects and exposure were wide (1.2-14.7). Also problematic is the observation that ethanol consumption during the first trimester was associated with a 2-fold lower malformation prevalence, suggesting a problem in methodology or sample size (Deane et al. 1989). Thus, the Santa Clara, California, studies are of limited value in addressing birth defects.

New Jersey

Bove et al. (1995) conducted a cross-sectional study of 80,938 births and 594 fetal deaths from 75 New Jersey towns, using records of water samples and birth and fetal death certificates for the calendar years 1985-1988. They estimated individual exposure information with information from the state monitoring program for multiple solvents. Analyses of water samples

detected trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane, 1,1- and 1,2-dichloroethylene, and at least 11 other solvents at <1 ppb. Decreases in adjusted mean birth weight of greater than 20 g were seen with trichloroethylene and total dichloroethylene exposure. An association was seen between exposure to trichloroethylene and low birth weight in term infants (OR = 1.23). No association was seen with small for gestational age or prematurity. Very low birth weight was associated with tetrachloroethylene exposure greater than 10 ppb (OR = 1.49). Fetal death was marginally associated with total dichloroethylene (OR = 1.18; 50% CI = 0.9, 1.55). For central nervous system defects, they found a positive association for total dichloroethylene exposure greater than 2 ppb (OR = 2.52, 90% CI = 1.25, 5.09). Neural tube defects were associated with total exposure to dichloroethylene (OR = 2.60, 90% CI = 0.93, 6.50) and were marginally associated with exposure to trichloroethylene greater than 10 ppb (OR = 2.53, 90% CI = 0.91, 6.37). However, the relationships between central nervous system and neural tube defects and trichloroethylene exposure were not monotonic, only the continuous variable was associated. In contrast to central nervous system anomalies, the relationship between trichloroethylene and oral clefts was monotonic if concentrations greater than 5 ppb were considered (OR = 2.24, 90% CI = 1.16, 4.20). Exposure to tetrachloroethylene was also associated with oral clefts (OR = 3.54, 90% CI = 1.28, 8.78). In a model that included other similar halogens, the OR for the association between oral clefts and exposure to trichloroethylene at greater than 5 ppb increased to 3.5, whereas that of other halogenated compounds fell with trichloroethylene exposure included. No relationship was seen between trichloroethylene and major cardiac defects or ventricular septal defects. This study likely includes a substantial amount of misclassification, which would decrease the power to detect a difference and would likely attenuate associations. The definition of small for gestational age as the smallest 5% would decrease power and prohibit comparison with other studies. In addition, effect modifiers were not assessed. Importantly, the extent of testing of interactions among solvents, other than the routine inclusion of total trihalomethanes in the analyses of trichloroethylene and similar compounds, is unclear. The passive ascertainment system used would likely yield valid results for easily detectable lesions such as oral clefts, but such systems are known to miss congenital heart disease (Cronk et al. 2003). The latter would again increase a type II error.

Tucson, Arizona

Three census tracts in Tucson, Arizona, (total population 1,099) were found to have trichloroethylene-contaminated well water between 1978 and 1981. Rodenbeck et al. (2000) estimated that concentrations of trichloroethylene in water ranged from less than 5 to 107 μg/L. Individual or household exposure could not be estimated because operational data were not available, so the entire population of all three tracts was considered evenly exposed. Mean exposure estimates were not given. Birth outcomes were compared between this group and contemporaneous births in other census tracts and for births in the census tracts after the exposure period (1983-1985). An association was reported between exposure to trichloroethylene via drinking water and very low birth weight (OR = 3.3; 95% CI = 0.5, 20.6). The authors suggested a similar association in the postexposure period; however, the magnitude was even smaller and less reliable (OR = 1.68, 95% CI = 0.41, 6.8). No relationship was seen between living in the exposed tracts and low birth weight or small-for-gestational-age babies. The problem of uncertain and uneven exposure is substantial and would decrease the power to

detect a difference. In addition, it is noteworthy that the exposure in this study was likely low compared with other population studies.

An increased frequency of congenital heart disease was suspected in Tucson, Arizona, in 1973. In 1981, drinking water contaminated with trichloroethylene (up to 270 ppb [approximately 0.009 mg/kg/day for a 60 kg adult], but also dichloroethylene and chromium) was detected in eight wells in Tucson Valley. In an epidemiologic study of children born between 1969 and 1987, Goldberg et al. (1990) noted that parents of children with congenital heart disease had a 3-fold greater likelihood of work or residence contact with the trichloroethylene-contaminated water area (n = 246/707, 35%) compared with parents of two "control" populations that had exposure rates of about 10%. The study has been criticized for inappropriate control groups, imprecision in determining exposure, and inclusion of years after the wells closed. Bove et al. (2002) reevaluated the data and restricted the analysis to the years when the wells were operational. In the reanalysis, the prevalence ratio of offspring cardiac defects among first-trimester "exposed" parents compared with that of "unexposed" parents was 2.58 (95% CI = 2.0, 3.4). Bove et al. (2002) also addressed the lack of exposure interviews for a large number of Goldberg et al. (1990) cases. Assuming that the noninterviewed cases and the interviewed cases had similar exposures or alternatively that the noninterviewed cases and the general Tucson population controls had similar exposures, the prevalence of cardiac defects in the exposed areas exceeded that in the uncontaminated areas by 2.3- and 2-fold, respectively. Thus, although the study by Goldberg et al. (1990) is flawed, additional analyses of the original data by an independent group of investigators yielded similar results and suggest an association between water contamination and congenital heart disease.

Milwaukee, Wisconsin

Yauck et al. (2004) performed a case-control study of 4,025 infants to evaluate the association between maternal residence close to trichloroethylene-emitting sites and infants with congenital heart defects in Milwaukee, Wisconsin. Mothers were categorized as older (older than 38 years) versus younger, exposed versus nonexposed, and presence versus absence of congenital heart defects. The proportion of mothers who were both older and had presumed trichloroethylene exposure was more than 6-fold greater among case infants (with congenital heart defects) than among control infants (3.3% versus 0.5%). When adjusted for other variables (e.g., race, ethnicity, maternal education, smoking), the risk of congenital heart defects was more than 3-fold greater among infants of older, exposed mothers than in infants of older, unexposed mothers (adjusted OR = 3.2; 95% CI = 1.2, 8.7). Older maternal age, alcohol use, chronic hypertension, and preexisting diabetes were each associated with increased incidence of congenital heart defects, but a residence close to trichloroethylene-emitting sites alone was not. The most common congenital heart defects were muscular ventricular septal defect (26.9%), secundum atrial septal defect (22.0%), membranous ventricular septal defect (20.8%), pulmonary stenosis (19.2%), and ventricular septal defect, not otherwise specified (15.5%). Maternal age was also an independent risk factor to other adverse birth outcomes, particularly chromosomal anomalies (e.g., Down's syndrome). Removing babies with any documented chromosomal abnormalities (n = 16) from the data set did not change the results of the logistic regression analysis.

Endicott, New York

The New York State Health Department in conjunction with ATSDR began an evaluation of health outcomes among residents living in areas of Endicott, New York, where soil vapor contamination with volatile organic compounds was identified (NYDOH 2005a). In the eastern study region, trichloroethylene was the most commonly found contaminant, occurring in indoor air at 0.18 to 140 $\mu g/m^3$, whereas reported soil values in some areas exceeded 10,000 $\mu g/m^3$. In the western study area, tetrachloroethylene was the most commonly found contaminant, ranging from 0.1 to 3.5 $\mu g/m^3$. The study years included 1978 to 2002 for the outcome variables birth weight and gestational age. Congenital anomalies were identified using the New York State Congenital Malformation Registry (data from 1983 to 2000). Individual information on each birth in the study and the comparison areas was used to estimate risk for each of the outcome variables, while controlling for maternal age, race, ethnicity, education and infant gender and year of birth.

When births (n = 1,440) in both study areas were considered together, the frequency of moderately low birth weight babies increased (standardized incidence rate [SIR] = 1.65 (95% CI = 1.00, 2.58) as well as term low-birth-weight births (SIR = 2.38; 95% CI = 1.10, 4.27). This observation was attributed to elevations observed in the eastern study region, the area with the greatest trichloroethylene contamination. In analyses that adjusted for multiple demographic factors, the relative risk of poor growth in the eastern study area was greater than in the controls. The ORs were 1.44 (95% CI = 1.13-1.83) and 1.79 (1.27-2.51) for low birth weight and term low birth weight, respectively. Among the congenital anomalies evaluated, the risk for all cardiac defects, as well as the subset of major cardiac defects, was elevated when both eastern and western areas were considered (adjusted rate ratio [RR] = 1.99; 95% CI = 1.27, 3.12; and RR = 2.62, 95% CI = 1.31, 5.23, respectively). Similar significant observations were seen for these end points when the eastern area was evaluated independently. The estimates from the data for the western study area were similar.

The evaluation of health effects at Endicott is an ongoing study and additional analyses and data refinements are planned. The current study is limited by the lack of individual exposure information, including concentration and duration of exposure. Birth defect cases were not validated by record review. Insufficient power was available to evaluate most birth defects. Finally, the quality of information for gestational age, a common problem with birth certificate data, was unclear but is needed for the subsequently planned study of small-for-gestational-age births.

Occupational Studies

Male Fertility

Bardodej and Vyskocil (1956) reported decreased libido in male workers exposed to trichloroethylene, but this effect did not appear to be related to any significant decrease in urinary excretion of adrenocorticosteroids. They gave no details about the control group, so the significance cannot be assessed. Sperm counts and morphology as well as Y chromosomal nondisjunction during spermatogenesis did not differ between male factory workers exposed to trichloroethylene at least 20 hours/week and physician controls (Rasmussen et al. 1988).

Chia et al. (1996) examined the effects of exposure to trichloroethylene on spermatogenesis among electronics factory workers. Of 450 men, 85 had seminal fluid samples analyzed within 2 hours of collection for seminal fluid volume, total sperm count, sperm viability, proportion of progressively motile sperm, and proportion of normal and abnormal sperm forms. Personal monitoring of 12 workers indicated that 11 were exposed to trichloroethylene at concentrations ranging from 9 to 26 ppm and 1 was exposed at 131 ppm. The geometric mean of the overall mean 8-hour exposure was 29.6 ppm, and the mean urinary trichloroacetic acid concentration was 22.4 mg per g of creatinine. Workers were divided into "high"- and "low"-exposure groups based on whether their normalized concentrations of trichloroacetic acid in urine was greater or less than 25 mg per g of creatinine, respectively. There were no differences between groups for any of the sperm parameters including volume, motility, and morphology; the values for both groups were within the standards of the World Health Organization (WHO). However, mean sperm density (million per mL) was increased in both groups relative to WHO norms, but the low-exposure group had higher sperm density than the high-exposure group. When the sperm densities were compared with urinary trichloroacetic acid quartile levels, the incidence of hyperspermia (>120 million per mL of ejaculate) increased with increasing urinary quartiles, consistent with a dose-response relationship. Although hyperzoospermia has been implicated in infertility, the authors were cautious about drawing a link, because no additional information has been reported about trichloroethylene and hyperzoospermia (Chia et al. 1996). When they analyzed the serum endocrine profiles in the same 85 male workers, Chia et al. (1997) found that the age of workers and years of exposure to trichloroethylene were significantly negatively correlated with testosterone concentrations. Years of exposure were also significantly positively correlated with dehydroepiandrosterone sulfate concentrations and negatively correlated with sex-hormone-binding globulin concentrations. Urinary trichloroacetic acid concentrations did not correlate with any hormonal measurement. When the men were stratified by years of exposure (<3, 3-5, 5-7, and ≥7 years), follicle-stimulating hormone was significantly reduced only in men exposed >7 years. Luteinizing hormone, testosterone, and free androgen index were statistically equivalent for all durations. Sex-hormone-binding globulin was significantly reduced only for a work duration of 5-7 years, and dehydroepiandrosterone sulfate concentrations were significantly increased for 3-5, 5-7, and >7 years. The workers had no clinical abnormality in reproductive function. The authors suggested that the reduction of follicle-stimulating hormone and testosterone could be due to disruption of peripheral endocrine function via trichloroethylene-induced reduction of liver production of sex-hormone-binding globulin and that chronic exposure to trichloroethylene also might have affected adrenal function.

A third report of the same workers evaluated whether trichloroethylene affected adrenal function (Goh et al. 1998). Contemporaneous blood samples were tested for testosterone, sex-hormone-binding globulin, androstenedione, cortisol, aldosterone, and insulin. Trichloroethylene did not significantly change adrenal steroid concentrations. Sex-hormone-binding globulin was significantly reduced for 4-6 years and for >6 years of trichloroethylene exposure. Insulin was significantly reduced among those with 2-4 or 4-6 years of exposure but not among those with >6 years of exposure. The authors concluded that urinary concentrations of trichloroacetic acid were significantly correlated with serum insulin concentrations. They further stated that insulin and sex-hormone-binding globulin "responded in tandem," with the highest concentrations in workers exposed less than 2 years and significantly reduced levels of

both parameters in workers exposed more than 2 years. They also described an unrealistic triphasic duration-dependent response in insulin concentrations to trichloroethylene.

In a more recent study, Forkert et al. (2003) examined eight mechanics with clinical infertility who had occupational exposure to trichloroethylene for at least 2 years. Seminal fluid from all eight subjects contained trichloroethylene, chloral, and trichloroethanol, whereas dichloroacetic acid and trichloroacetic acid were present in only two and one sample, respectively. Neither trichloroethylene nor its metabolites was detected in the five control male seminal fluid samples. CYP2E1 was found in normal human testes and epididymides, specifically in the Leydig cells in the interstitium of the testis and the caput (head), corpus (body), and cauda (tail) of the epididymis. This finding was consistent with findings in rodents, which demonstrated that CYP2E1 was involved in trichloroethylene metabolism in these tissues (see earlier discussion of studies on rodents and nonhuman primates, including those by Forkert et al. 2002, 2003).

Pregnancy Outcomes

McMartin et al. (1998) performed a meta-analysis of five retrospective studies evaluating pregnancy outcome after maternal exposure to organic solvents (Eskenazi et al. 1988; Lipscomb et al. 1991; Windham et al. 1991). Sample sizes ranged from 570 to 2,950 to yield a total of 7,036 pregnancies. They selected studies for inclusion if the outcomes included spontaneous abortion before 20 weeks, and they were either case-control or cohort studies that involved first-trimester maternal inhalational exposure to organic solvents in an occupational setting. The results indicated a small, equivocally significant effect of occupational exposure on spontaneous abortion (summary OR = 1.25, 95% CI = 0.99, 1.58). The summary OR was higher (1.54; 95% CI = 1.07, 2.21) when an unpublished study of the solvent styrene was removed from the analysis. Results of the subanalysis of the cohort studies and the case-control studies were fairly similar, as were the results when unpublished studies were excluded. However, the implications for trichloroethylene per se are impossible to know, as the definition of solvent was very broad and ill-defined.

In a later prospective study, 125 pregnant women occupationally exposed to solvents were compared with 125 unexposed women matched for age, gravidity, smoking, and alcohol histories (Khattak et al. 1999). Significantly more malformations occurred among fetuses of exposed than unexposed pregnant women (RR = 13, 95% CI = 1.9, 99.5). Major malformations were more common among women who prospectively reported temporally associated exposure symptoms (eye and respiratory irritation) than among occupationally exposed, but asymptomatic, women (12/75 versus 0/43; $P < 0.001$). No pattern of association was detected in this small study of a mixture compounds. Exposed women were more likely to have had previous miscarriages, but the rates of malformations were similar among exposed women who did and did not have histories of miscarriage.

Taskinen et al. (1989) conducted a case-control study nested in an occupational cohort who were monitored biologically for exposure to six organic solvents, including tetrachloroethylene, trichloroethylene, and 1,1,1-trichloroethane. Biological samples (n = 13,132) were obtained from approximately 6,000 men. Spontaneous abortion was associated with increased paternal exposure to solvents in general (adjusted OR = 2.3, 95% CI = 1.1, 5.0). They observed no association between paternal halogenated hydrocarbon exposure as a class or

with exposure to tetrachloroethylene, trichloroethylene, or 1,1,1-trichloroethane. However, the total number of fathers exposed to any halogenated hydrocarbon was 92 (31 cases and 61 referents). The sample size was too small to test associations between organic solvents and birth defects.

In another study of women who were biologically monitored (8,547 samples from 3,265 women) for exposure to solvents, Lindbohm et al. (1990) found that exposure to solvents was more common among women who had spontaneous abortions than in controls (adjusted OR = 2.2, 95% CI = 1.2, 4.1). The sample size for assessing individual solvents was small; 42 subjects were exposed to halogenated hydrocarbons (n = 14 cases, 28 controls). The ORs for associations between spontaneous abortion and exposure to individual solvents were 1.4 (95% CI = 0.5, 4.2) for tetrachloroethylene, 0.6 (95% CI = 0.2, 2.3) for trichloroethylene, and 3.4 (95% CI = 0.7, 16.9) for 1,1,1-trichloroethane. No clearly significant associations were seen for any of these solvents.

In a prospective study of 3,216 pregnant women, no association was seen between exposure to organic solvents and having a small-for-gestational-age offspring (Seidler et al. 1999). However, the exposure assessment used a relatively crude exposure tool, the Pannett job exposure matrix, and exposure to organic solvents was low. No women reported high exposure; only 23 and 73 women reported moderate and low exposure, respectively. In addition, selection bias occurred; recruitment methods decreased the likelihood that higher-risk pregnancies were included.

Using a multicenter European case-control study with six congenital malformation registries between 1989 and 1992, Lorente et al. (2000) examined occupational exposures of women (100 mothers of babies with oral clefts and 751 mothers of healthy babies) who worked during their first trimester of pregnancy. After adjustment for potential confounding factors (such as center of recruitment, maternal age, urbanization, socioeconomic status, and country of origin), only cleft palate was significantly associated with maternal occupation in services such as hair dressing (OR = 5.1, 95% CI = 1.0, 26.0) or housekeeping (OR = 2.8, 95% CI = 1.1, 7.2). The analyses further suggested that several occupational exposures were associated with orofacial clefts. Cleft palate as an isolated anomaly was associated with trichloroethylene exposure (OR = 6.7, 95% CI = 0.9, 49.7). Furthermore, among patients with cleft palate only, the risk increased with the concentration and frequency of trichloroethylene exposure. For low exposure to trichloroethylene, the OR was 6.6 (95% CI = 0.6, 79); for medium exposure it was 13.9 (95% CI = 1.1, 186). Because oral clefts are among the most frequent congenital anomalies (with a prevalence in Europe of 1 in 700 births) and are multifactorial in origin, and because this study involved a limited number of subjects, the results should be interpreted with caution.

Shaw et al. (1992) reported the findings of a case-control study of congenital heart disease among Santa Clara County births during the calendar years 1981-1983. Mothers of cases (n = 141) were more likely to report occupations associated with organic solvent exposures than mothers of controls (OR = 1.8, 95% CI = 0.95, 3.3). Problems with this study include heterogeneity of cardiac defects, the likelihood of recall bias, the exclusion of some lesions, and, importantly, the fact that exposure to contaminated drinking water was not included, even though it was known that county residents were exposed to trichloroethylene-contaminated water during part of the study period.

In the large Baltimore-Washington study of congenital heart disease, exposure to degreasing solvents (such as trichloroethylene), was 8 and 12 times more likely among mothers of infants with left-sided flow-obstructive lesions and aortic stenosis, respectively (Loffredo et

al. 1991). Other, less robust, human epidemiologic studies have evaluated occupations likely to involve exposure to solvents. In these studies, mothers of infants with congenital heart disease were roughly twice as likely as controls to have exposures to organic solvents (McDonald et al. 1987; Tikkanen and Heinonen 1988).

FINDINGS AND RECOMMENDATIONS

The fundamental question for this chapter is whether there is necessary and sufficient evidence from the animal and epidemiologic studies that trichloroethylene, at environmentally relevant doses or concentrations, causes adverse effects on reproduction or birth outcomes. In synthesizing the large body of literature addressing developmental and reproductive toxicity, the committee identified those end points for which the animal and human evidence generates the greatest level of plausibility. These end points are discussed below and include impaired intrauterine growth, cardiac teratogenesis, and altered spermatogenesis. Although the evidence suggests that trichloroethylene can generate such effects, the lowest-observed-adverse-effect level for human risk assessment remains unclear. Some information suggests that certain human subpopulations might be at increased risk because age, genetic polymorphisms, or disease (see Chapter 9). Selection of these three end points indicates not that other reproductive or developmental end points do not have an association with trichloroethylene, but rather that the combined human and animal evidence generated to date does not reach levels of reasonable plausibility.

Intrauterine Growth

The collective data on the developmental toxicity of trichloroethylene provide substantial evidence that trichloroethylene in drinking water might cause impaired intrauterine growth at environmentally relevant concentrations. Substantial decreases in fetal growth were found among offspring of women who lived in areas of Camp Lejuene, North Carolina, with contaminated water systems (ATSDR 1998). Plausibility is increased by the observation that longer exposure was associated with a marked decrease in birth weight. This observation is replicated in an exposed population in New Jersey (Bove et al. 1995), albeit with a smaller, though statistically significant, diminution in birth weight. Furthermore, a statistical decrease in birth weight was seen among offspring whose mothers were exposed to tetrachloroethylene (Sonnenfeld et al. 2001). A recent ATSDR report found decreased intrauterine growth in mothers who lived in areas with trichloroethylene and tetrachloroethylene contamination (NY Department of Health, ATSDR report). In addition, the association between increased risk of poor fetal growth among older mothers exposed to tetrachloroethylene is similar to that of other solvents, such as ethanol, increasing the plausibility of this observation. In animal studies, decreased intrauterine growth after maternal trichloroethylene exposure has been found consistently (Bross et al. 1983; Smith et al. 1989, 1992; Johnson et al. 1998a,b; Fisher et al. 2001). However, in rodent studies, dichloroacetic acid at doses as low as 140 mg/kg/day was associated with this effect (Smith et al. 1992).

Recommendation: Additional studies to delineate subpopulations at greatest risk as well as to determine the mechanisms for the putative gender and maternal age-based susceptibility are warranted. Such interactions might be confirmed with analysis of existing epidemiologic data sets.

Cardiac Teratogenicity

Cardiac teratogenicity is the developmental end point in animal studies that has received the greatest attention. The committee is aware that considerable controversy has existed regarding cardiac teratogenesis, with some reviewers on both sides of the argument (Kaneko et al. 1997; Johnson et al. 1998b; Bove et al. 2002; Hardin et al. 2005). Multiple studies in several animal models, including mammalian (Smith et al. 1989, 1992; Epstein et al. 1992; Dawson et al. 1993; Drake et al. 2006) and avian (Bross et al. 1983; Loeber et al. 1988), suggest that trichloroethylene, or one or more of its metabolites (trichloroacetic acid and dichloroacetic acid), can cause cardiac teratogenesis. Of the studies performed, the avian studies are the most convincing, and mechanistic studies in birds have been performed. Although some rodent studies have shown effects (Smith et al. 1989, 1992; Dawson et al. 1993; Epstein et al. 1992), other studies have not (NTP 1985, 1986b; Fisher et al. 2001), suggesting either methodological or strain differences. The committee noted that the rodent studies showing trichloroethylene-induced cardiac teratogenesis at low doses were performed by investigators from a single institution. Also noted were the unusually flat dose-response curves in the low-dose studies from these investigators. For example, the incidences of heart malformations at trichloroethylene concentrations of 1.5 and 1,100 ppm (almost three orders of magnitude greater) were 8.2% to 9.2% (prepregnancy and during pregnancy) to 10.4% (during pregnancy only) (Dawson et al. 1993). The same pattern occurred with dichloroethylene. Thus, the animal data are inconsistent, and the apparent species differences have not been addressed.

Of the human epidemiologic studies, the Bove et al. (2002) reanalysis of the widely criticized, but positive, study by Goldberg et al. (1990) also found a positive association. Methodological problems limited the committee's consideration of the Santa Clara County data for congenital heart disease. The recent report of an increased incidence among residents of the Endicott, New York, area was also consistent with the Goldberg study. Of note, the effect size of a 2- to 3-fold increase in risk is similar across multiple studies. Plausibility for trichloroethylene-induced cardiac teratogenesis is increased by the fact that the most frequently observed cardiac defects in the human studies, those of the interventricular septae and the valves, are consistent with the most common defects seen in the animal studies. In addition, these specific defects are consistent with mechanistic studies demonstrating altered increased proliferation in the endocardial cushions at low dose (Drake et al. 2006) or alterations in endothelial cell activation and decreased expression of the transcription factor Mox-1 and extracellular matrix protein fibrillin 2, two markers of epithelial mesenchymal cell transformation, a key process in valve and septum formation (Boyer et al. 2000). Evidence that trichloroacetic acid and dichloroacetic acid are as potent as the parent compound suggests that CYP2E1 metabolic activation, as well as the fractional formation of trichloroacetic acid from chloral, is important in trichloroethylene cardiac teratogenesis.

Recommendations: Additional studies evaluating a lowest-observed-adverse-effect level and mode of action for trichloroethylene-induced developmental effects are needed to determine the most appropriate species for human modeling. More information is needed on metabolic activation in the avian model to evaluate interspecies differences, tissue-specific concentrations of trichloroethylene and its metabolites, and human data with better ascertainment of congenital heart disease and improved quantitative assessment of trichloroethylene exposures. Reanalysis, or perhaps additional data collection, from previous epidemiologic studies could be performed. For example, for some studies, more appropriate control data might be derived, which would cost-effectively improve the assessment of human trichloroethylene teratogenesis. The interaction of trichloroethylene with other solvents, some of which are known teratogens (e.g., ethanol and toluene), might also be pursued.

Reproductive Toxicity

On the basis of evidence generated by multiple authors in multiple rodent studies (Land et al. 1981; Kumar et al. 2000a; Forkert et al. 2002), the committee suggests that trichloroethylene is toxic to spermatogenesis and sperm fertilizing ability. However, whether these effects are transient or permanent is unclear. The mode of action is unclear and might or might not relate to hormonal alterations. Critical work by Berger and Horner (2003) demonstrated that trichloroethylene and tetrachloroethylene are not only male reproductive toxicants but also female reproductive toxicants in rats. Evidence for this finding included decreased sperm penetration and decreased fertilizability of oocytes from trichloroethylene- and tetrachloroethylene-treated females and reduced sperm plasma membrane protein binding to oocytes from trichloroethylene-treated females. Metabolic activation by CYP2E1 appears necessary for toxicity; however, which of the oxidative downstream metabolites is the proximate toxicant is not yet clear. The relevance of these trichloroethylene effects on male and female reproduction in animals to adverse reproductive outcomes in humans also is not clear.

Recommendations: More research is needed to better understand the effects of trichloroethylene on sperm and oocytes and possible consequences for reproduction. Mechanistic studies are needed to determine what metabolites are responsible for the effects.

6

Neurotoxicity

This chapter comments on the discussion of neurotoxicity in the U.S. Environmental Protection Agency (EPA 2001b) draft risk assessment of trichloroethylene and reviews information on the effects of trichloroethylene on the nervous system generated since that document was released. Other recent reviews are considered, including those of the Agency for Toxic Substances and Disease Registry (ATSDR 1997a) and the New York State Department of Health (NYDOH 2005). The chapter also addresses (1) information about the effects on complex cognitive functions, (2) sensitive populations, (3) known interactions of trichloroethylene with other exposures that may affect the risk for neurotoxicity, (4) the role of trichloroethylene concentrations in the brain, (5) the potential role of trichloroethylene in the development of neurodegenerative diseases, (6) potential mechanisms of effect and their implications for complex behavioral function, and (7) research needs.

BACKGROUND

In the past, trichloroethylene was widely used as an anesthetic at concentrations of approximately 2,000 parts per million (ppm). That use was generally restricted around 1977 because of adverse effects associated with such treatments (ATSDR 1997a). Given trichloroethylene's anesthetic uses and its widespread use in occupational settings, significant information is available on the acute toxicity of trichloroethylene and its metabolites. Surprisingly, little information exists on the effects of more protracted exposures on the central nervous system, either in humans or in experimental models, particularly at lower concentrations of exposure. Where studies are available, information from human populations often relies on estimated rather than actual concentrations of exposure, making it difficult to evaluate risks to health. In addition, much of the literature related to trichloroethylene exposure in humans includes exposures to mixtures of solvents, so that it is difficult to evaluate the specific contribution of trichloroethylene to health outcomes.

ANIMAL TOXICITY

Acute Exposure

Experimental studies of acute exposures in rats have shown behavioral alterations across several functional domains at a range of concentrations that overlap with those associated with effects in humans. Most of these studies involved inhalation exposures. At higher concentrations of exposure (e.g., 1,000-4,000 ppm), reported effects include hearing loss, impaired oculomotor control, seizures, decreased wakefulness, and anesthetic effects such as lethargy and ataxia.

Auditory deficits have been observed in several studies at comparable exposure concentrations and in different strains of rats, attesting to the generality of the effects. These studies show auditory effects to occur primarily for the midfrequency tone range (Mattsson et al. 1993; Crofton and Zhao 1993; Jaspers et al. 1993; Rebert et al. 1993; Crofton et al. 1994). Studies have indicated the persistence of some adverse auditory effects, as evidenced 14 weeks postexpsoure to trichloroethylene at 4,000 ppm for 6 hr/day for 5 days (Crofton and Zhao 1993). Apparently, this outcome has not been studied after acute exposures of humans to high concentrations of trichloroethylene.

In rat models, high doses of trichloroethylene administered orally (2,500 mg/kg per day, 5 days per week for 10 weeks) result in morphologic changes in nerves, including alterations in myelination characteristics of the trigeminal nerve (Barret et al. 1991, 1992). These findings are consistent with reports of cranial nerve damage in humans (e.g., Cavanagh and Buxton 1989). A role was noted for the trichloroethylene degradation byproduct dichloroacetylene in eliciting these effects.

Effects on behavior at lower trichloroethylene concentrations in experimental studies have included impaired effortful motor response in rodents (measured by swimming performance) and decreased response of rats to avoid electric shock after a 4-hour exposure to trichloroethylene at 250 ppm (Kishi et al. 1993). The concentrations at which Kishi et al. (1993) observed effects are similar to those noted by Stewart et al. (1970) in humans reporting headaches, fatigue, and drowsiness after exposure to trichloroethylene for 7 hr/day for 5 days. ATSDR (1997a) used these studies to formulate a minimum risk level for acute duration inhalation in humans.

A newer study by Ohta et al. (2001), not available at the time of the EPA or the ATSDR review, examined the effects of trichloroethylene on long-term potentiation (an enduring increase in the efficacy of specific brain pathways), one of the hypothesized neurophysiologic mechanisms for learning. They evaluated measurements of long-term potentiation in hippocampal slices in mice 24 hours after single exposures to trichloroethylene. They observed dose-related decreases in potentiation of the action potentials of a population of neurons (population spikes) after tetanus treatment, with reductions of 15% at 300 mg/kg and of 26% at 1,000 mg/kg. The size of the area responsive to potentiation was also reduced by trichloroethylene exposure. The animals did not appear to be anesthetized by this dose. One difficulty in comparing exposures for the effects observed by Ohta et al. (2001) with those from other studies of acute exposures is the difference in route of exposure. Ohta et al. (2001) used intraperitoneal injections and did not provide any information about peak brain concentrations of trichloroethylene produced by this exposure. However, efforts should be made to estimate from physiologically based pharmacokinetic models what the peak brain concentrations would be in

this study and how they might compare with other routes for potential utility in evaluating acute-exposure risk assessment, given the nature and magnitude of the reported effect and its observation 24-hours post-exposure.

Significantly more information is available with regard to inhalational exposures in rats. Many studies have focused on sensory-based alterations in response to trichloroethylene. Reported effects include changes in the amplitude of flash-evoked potentials (visual function) (Blain et al. 1992; Albee et al. 1993), reduced acoustic startle response, and auditory-evoked potentials (auditory function) (Rebert et al. 1991; Jaspers et al. 1993), consistent with the auditory effects described above. As with higher concentrations, lower concentrations alter the shock-avoidance response (Goldberg et al. 1964).

Intermediate-Duration Exposure

Among studies using intermediate subchronic exposures, the lowest concentration of trichloroethylene associated with effects on the nervous system comes from a report by Arito et al. (1994). Rats exposed for 8 hours a day, 5 days a week for 6 weeks showed decreased wakefulness and increased slow-wave sleep during the period of exposure. When measured 22 hours after exposure, the rats showed decreased heart rates during sleep. The effects on wakefulness and sleep were observed at exposures of 50 ppm, as well as at 100 and 300 ppm, and were not dose related. Moreover, they persisted over the 6 weeks of the study (no adaptation was observed). These effects might relate to the fatigue and lethargy associated with exposure to trichloroethylene in human studies. ATSDR (1997a) used data from the 50-ppm exposure to trichloroethylene by Arito et al. (1994) to determine an intermediate-duration inhalation minimal risk level of 0.1 ppm. EPA (2001b) used a lowest-observed-adverse-effect level (LOAEL) of 50 ppm from this study to derive a pharmacokinetic-adjusted human equivalent concentration of 9 ppm and a benchmark dose associated with a 10% response (BMD_{10}) of 5 ppm. These levels of effects are directly comparable to LOAEL values determined from human studies based on chronic exposures (described later in this chapter).

Isaacson and Taylor (1989) studied the effects of trichloroethylene on rats exposed during development (gestation and lactation) at concentrations of 312, 625, and 1,250 mg/L in drinking water. These exposures were reported to increase exploratory behavior in 60- and 90-day-old offspring, with the highest exposure concentration increasing locomotor activity at 60 days of age. These exposures likewise resulted in a 40% reduction in the number of myelinated fibers in the hippocampus, a region critical to complex cognitive function (both 312 and 625 mg/L or, equivalently, 4.0 and 8.1 mg of trichloroethylene per day, respectively). It is not known, however, whether these concentrations were associated with effects on maternal weight gain during pregnancy or on litter size and pup brain and body weights. Also, doses to the pups are not known, making extrapolation difficult. Nevertheless, these effects speak to the potential for permanent damage resulting from trichloroethylene exposure during development.

Studies in gerbils reported changes in the expression of protein concentrations in the brain at lower concentrations of trichloroethylene. This includes inhalation exposures to trichloroethylene at 60 or 320 ppm for 3 months, followed by a 4-month postrecovery period, after which increases in proteins appeared in multiple brain regions, even in response to the lower concentration. DNA was elevated in two regions at 320 ppm, with a LOAEL of 60 ppm (Haglid et al. 1981). A decrease in S100 protein concentrations, thought to be a marker of brain

damage, was observed after exposure to trichloroethylene at 170 ppm or after intermittent exposure at 500 ppm for 5 months, with no postexposure recovery period (Kyrklund et al. 1984). These seemingly opposite effects of exposure to trichloroethylene could reflect the dynamics of the protein response over the period of exposure and recovery rather than discrepancies in outcome. Although these findings are potentially interesting, it is difficult to extrapolate from gerbils to humans, because kinetic characteristics of trichloroethylene in gerbils are unknown relative to rats and mice and there have been no follow-up reports in rats or mice.

Studies of subchronic exposure to trichloroethylene in rats reported after the EPA (2001b) draft risk assessment include that of Poon et al. (2002) and Oshiro et al. (2004). Poon et al. (2002) examined the effects of oral exposures to trichloroethylene at 0, 0.2, 2, 20, and 200 ppm in male and female Sprague-Dawley rats for 13 weeks. Of relevance to neurotoxicity were measures of histologic changes in the myelin sheath of the optic nerves and concentrations of biogenic amines, determined in several different brain regions in a subset of males. In the absence of reductions in food or water intake or in body weight gain, the authors reported a mild vacuolation of the myelin sheath at the highest concentration (200 ppm) in 30%-70% of the animals examined. However, there was no associated axonal degeneration or lymphoid infiltration, making interpretation of these findings difficult. It was not clear how many animals were examined for this effect, how the 30%-70% range was determined, or whether these tissues were examined in a blinded fashion. Because measurement was taken at a single time point during the exposure, it is difficult to determine the degree of damage it signifies and whether such effects were progressive with time or represent early exposure effects. No changes in biogenic amines were reported by Poon et al. (2002), as examined in frontal cortex, caudate nucleus, nucleus accumbens, hippocampus, and substantia nigra. However, the sample sizes ($n = 5$) used for this component of the study compromise the ability to detect such changes, which normally would require sample sizes up to double those used here. This problem is clear from the measures of variability presented for these data, with standard deviations as high as 50% for the control group in some cases. Thus, the general absence of effects in this study may reflect experimental parameters rather than an insensitivity of the nervous system to these concentrations of trichloroethylene. Given these limitations, the utility of these data for risk assessment is questionable despite the focus on low doses of trichloroethylene.

It appears that repeated exposures to trichloroethylene can impair sustained attention but do not seem to produce a residual impairment in this behavioral process. Bushnell and Oshiro (2000) reported that exposures to trichloroethylene at 2,000 or 2,400 ppm via inhalation for 9 days disrupted performance on a sustained attention task, decreasing the probability of a hit (correct response to a signal) and increasing response time as well as the number of response failures. Tolerance to these impairments developed over the course of exposure, however, and additional work is required to determine whether this reflected metabolic or behavioral tolerance.

In a follow-up study, Oshiro et al. (2004) examined the residual neurological effects of exposure to trichloroethylene at 0, 1,600, or 2,400 ppm for 6 hours a day for 20 days in adult male rats, with evaluation of learning a sustained attention task beginning 3 weeks postexposure. No exposure-related effects were found. Both ethanol and d-amphetamine impaired performance on the task, with a more pronounced reduction of the probability of a correct response to a signal at the highest dose of amphetamine in the group exposed to trichloroethylene at 2,400 ppm. The authors suggested that the discrepancy between the findings of this study and those of human studies that found residual deficits in cognitive function might be due to differences in the duration and number of exposures to trichloroethylene. Human studies that found residual

effects involved exposure durations ranging from a mean of 3 years to 24.5 years, whereas the exposures in the Oshiro et al. study were estimated to be equivalent to 2.6 and 3.8 years of exposure for humans. Furthermore, many previous reports of attention effects reflect exposures to mixtures of solvents rather than trichloroethylene alone, raising questions about the specific components of the exposures that would have contributed to the effects.

Although these findings suggest no residual learning deficits after trichloroethylene exposure, the differential effects of amphetamine in control versus trichloroethylene-treated animals could indicate residual effects on brain dopamine neurotransmitter systems after trichloroethylene exposure. Dopamine pathways of the central nervous system are critical for cognitive and executive functions. Moreover, they further support the potential for trichloroethylene to have protracted effects even after exposure ceases.

Waseem et al. (2001) examined neurobehavioral effects of trichloroethylene in rats after oral administration for 90 days of 350, 700, or 1,400 ppm or after inhalation exposure of 376 ppm for 4 hours per day, 5 days per week, for a total of 180 days. Locomotor activity was measured in addition to "cognition" which was evaluated using acquisition of a conditioned shock-avoidance response. Neither oral nor inhalation exposure resulted in differential effects on acquisition of the response as measured for 7 days immediately after 90 days of oral exposure or 180 days of inhalation exposure. One problem with interpretation of these studies was the experimental design in which acquisition was studied after trichloroethylene exposure. The acquisition of shock avoidance is critically dependent on the intensity of the shock stimulus. It is possible that exposure to trichloroethylene altered shock sensitivity per se. If shock sensitivity were actually reduced, one might expect a lower rate of acquisition. Thus, rates of acquisition would have to be "normalized" to shock sensitivity. Differences in sensitivity to shock per se were never compared between the two groups. In addition, rats exposed to trichloroethylene had higher levels of locomotor activity. The increased levels of motor activity also could have contributed to levels of shock avoidance, causing higher levels of movement between the chambers of the shuttle box used to measure avoidance. Although these increases were stated to be significant at days 30 and 90 of exposure and not statistically significant at day 180, the trends were still evident at 180 days, and the small number of animals used in the experiments (six per group) would likely have precluded the ability to statistically confirm such differences, particularly as locomotor activity varies substantially among animals. For these reasons, it is not possible to determine whether there were differences in learning between trichloroethylene-exposed and control animals in these experiments. Even if there were differences, the values here were not below current LOAEL values.

Chronic Exposure

Few experimental studies examining the effects of chronic exposure to trichloroethylene (>365 days) have been reported. In one study (NTP 1988), rats were administered trichloroethylene at 500 or 1,000 mg/kg per day for 103 weeks via gavage. The report described (but did not quantify) transient postdosing effects in rats that are consistent with previous reports in human and experimental exposures (lethargy, ataxia, and convulsions). One notable observation from that study suggesting a sensitization effect was that some convulsions occurred before dosing, during the weighing period. A study of mice exposed for 54 weeks to 2,400 mg/kg per day (males) or to 1,800 mg/kg per day (females) reported nonquantified observations

of excitation immediately after dosing followed by anesthetic-type effects (Henschler et al. 1984). These reports indicate a consistency of trichloroethylene's effects across species but do not provide information of particular use to the risk assessment process. Moreover, these studies examined relatively high concentrations of trichloroethylene as they were carried out in the context of carcinogenicity evaluations.

Not surprisingly, given its anesthetic properties, chronic exposures to trichloroethylene have been shown to alter neurotransmitter functions. In a study of gerbils exposed to trichloroethylene for 12 months via inhalation at 50 and 150 ppm, dose-dependent increases (52% and 97% for glutamate; 69% and 74% for γ-aminobutyric acid [GABA], respectively) were observed in the uptake of glutamate and GABA in the posterior cerebellar vermis but not in the hippocampus; perchloroethylene did not produce corresponding changes, suggesting some specificity of the effect (Briving et al. 1986). These effects were seen in the absence of any changes in body or whole brain weights and so do not appear to reflect systemic toxicity. The LOAEL for this study was 50 ppm. Difficulties in using these data include extrapolation to human exposure, given differences between the gerbil and more standard rat and mouse models for which toxicokinetic parameters are well described. Nevertheless, they appear to support the EPA-derived LOAEL of 50 ppm from Arito et al. (1994).

HUMAN TOXICITY

Acute Exposure

Effects from acute (<14 days) exposure to trichloroethylene are widely reported in humans. At lower exposures (50-300 ppm), headache, fatigue, drowsiness, and inability to concentrate are reported. As the trichloroethylene concentrations increase, dizziness, loss of facial sensation and unconsciousness can occur. With acute exposures to high concentrations (albeit highly unspecified [e.g., 1,000 ppm and above; anesthetic use was approximately 2,000 ppm]), trichloroethylene has been associated with dizziness, headache, euphoria, sleepiness, nausea, confusion, and visual and motor disturbances. Acute exposures to high concentrations, most often due to accidental occupational exposures at unspecified concentrations, have been associated with nerve damage (typically cranial nerves) and residual neurological deficits, including memory loss when measured as long as 12-18 years later (e.g., Feldman et al. 1985). A remaining uncertainty is whether the reported nerve damage results from trichloroethylene or from a metabolite.

In many reports, actual concentrations of trichloroethylene are unspecified. In a controlled exposure experiment using trichloroethylene at 100 ppm for 6 hours a day for 5 consecutive days, Triebig et al. (1977) reported no statistically significant differences between exposed and control subjects in standardized achievement tests and self-reporting scales. Stewart et al. (1970) used human volunteers exposed to trichloroethylene at defined concentrations for specified durations to evaluate changes in motor function. Outcomes in these tests were normal in response to 200 ppm, but subjects complained of fatigue and drowsiness as well as a need to exert greater mental effort on the tests, an effect that may reflect the symptoms described. In a review of the literature, ATSDR (1997a) used the study by Stewart et al. (1970) to derive an acute-duration inhalation minimum risk level. This was later adjusted to determine an intermediate-duration inhalation minimal risk level of 0.1 ppm.

Intermediate Chronic Duration Exposure

The assessment of intermediate subchronic exposures (15-364 days) reviewed by both ATSDR (1997a) and EPA (2001b) focused on studies in rats because most human studies involved chronic exposures. In general, effects from intermediate subchronic exposures are similar to those reported at higher concentrations but occur at lower trichloroethylene concentrations with more protracted duration exposures.

In a review of the published literature, ATSDR (1997a) did not cite any long-term exposure studies in humans, because exposures to trichloroethylene in these studies are unspecified (estimated rather than empirically measured). EPA (2001b) adopted a different approach, validly recognizing the adverse effects reported for humans experiencing chronic exposure. The approach reflects a weight of evidence of effects from low-dose exposures that included adverse outcomes on the central nervous system, as well as other target organs, and involved development of reference doses and reference concentrations (RfCs) and pharmacokinetic modeling. Thus, in its evaluation, EPA was more inclusive in its use of information related to longer-term exposure.

Studies of central nervous system toxicity identified by EPA include effects that are also reported in response to shorter durations and higher concentrations of trichloroethylene and, thus, represent a continuum of these effects along the dose and exposure duration-response curve, with trichloroethylene effects appearing at lower concentrations when exposure durations are longer. In addition, the LOAELs from four different animal studies examining nervous system effects show a high degree of correspondence, ranging from 20 to 50 ppm, with corresponding human equivalent concentrations of 7-16 ppm.

Among the human studies, the report by Ruijten et al. (1991) demonstrates changes in trigeminal nerve function measured using the massiter reflex latency in workers whose exposure was below the threshold limit value (35 ppm) and whose exposure duration averaged 16 years. These findings correspond to reports from studies cited above of trigeminal and cranial nerve damage at shorter-duration exposure to high concentrations of trichloroethylene. In addition, this study noted slight reductions in the sensory nerve conduction velocity and the sensory refractory period of the sural nerve, consistent with preclinical peripheral nervous system impairments and with corresponding reports from experimental studies. Because all workers had been employed as printers in the same workplace and factory, the exposures were likely highly homogenous within this group. A human equivalent concentration LOAEL of 16 ppm was determined by EPA from this report using RfC methodology for a category 3 gas, extrathoracic effects (EPA 1994b).

Rasmussen et al. (1993) examined changes in cranial nerve function, motor coordination, and vibration sensitivity in metal degreasers whose primary exposure was to trichloroethylene (determined from biomonitoring data from the Danish Labour Inspection Service). Exposure durations were shorter than in the Ruijten et al. (1991) study. Highly significant dose-related increases were seen in motor dyscoordination. These findings are impressive because they were measured by clinical neurological examination, a far less sensitive approach than is available with more sophisticated technologies (e.g., Weiss and Cory-Slechta 2001), although it is not clear whether the examiner was blind to the exposure categorization of each worker. Abnormal olfactory (cranial nerve) function was also dose related, with similar, but not significant, trends for trigeminal nerve sensory function and facial nerve function measured via taste. The human

equivalent LOAEL determined by EPA from these studies was 7 ppm using RfC methodology for a category 3 gas, extrathoracic effects (EPA 1994b).

These reports are further supported by early studies reporting symptoms of drowsiness, fatigue, headaches, and nausea in response to occupational inhalation exposures to trichloroethylene over a mean of 7-8 years, with human pharmacokinetic adjusted LOAELs using RfC methodology for a category 3 gas, extrathoracic effects (EPA 1994b), of 7-11 ppm (Okawa and Bodner 1973; Vandervort and Polakoff 1973). Moreover, all four human studies demonstrated effects in the same exposure range as the report of decreased wakefulness by Arito et al. (1994) in rats after subchronic inhalation exposures (LOAEL of 50 ppm with human pharmacokinetic adjusted value of 9 ppm and a human pharmacokinetic adjusted BMD_{10} value of 5 ppm).

Three new human chronic exposure studies have appeared since the reviews by ATSDR and EPA. Two of them examine the impact of environmental trichloroethylene exposures on neurobehavioral function (Kilburn 2002a,b). Exposures were estimated based on groundwater plumes measured during a 3-month period. Concentrations of trichloroethylene measured in well water ranged from 0.2 to 10,000 parts per billion (ppb). Exposed subjects (n = 236) lived near two electronic manufacturing plants and were involved in litigation related to these exposures; referents matched on a number of other factors (n = 161) lived in a town without contaminated water located 88 km upwind from the exposed subjects. Additional reference subjects (n = 67) were from the same geographic area as the subjects but had never lived in the exposure zone. In the first of these studies, exposed subjects were reported to have delayed simple and choice reaction times; impaired balance; delayed blink reflex latency; abnormal color discrimination; and impaired cognitive function, attention, recall, and perceptual speed (Kilburn 2002a). This study has many limitations. Exposures are estimated, not directly measured, and involve mixed solvent exposures (although the author states that the primary toxicant was trichloroethylene), examiners did not appear to have been fully blinded to treatment conditions, and the period when trichloroethylene was measured was brief. Further, subjects were involved in a lawsuit related to this exposure, introducing the potential for bias. In addition, all relevant comparisons were not made (e.g., the two reference groups were never shown to be comparable), and the pattern of differences between referents and subjects was not the same in the two groups (e.g., subjects versus local referent outcomes was not the same as subjects versus referents living 88 km distant). Thus, the reliability and utility of these findings is questionable and their relationship to exposure levels is unknown.

A second study published by the same author attempted to address the issue of the potential bias introduced by the subjects being involved in ongoing litigation (Kilburn 2002b). In this case, the 236 subjects were compared with 58 nonclaimants within the three residential areas in the exposure zone. In addition, subjects were divided into two groups based on duration of exposure (and, presumably, years of litigation as well). In addition to having the same study limitations noted above, other inconsistencies were noted. For example, subjects with shorter exposure durations to trichloroethylene had significantly abnormal sway (balance) relative to subjects with longer exposures, despite the fact that they were also 10 years younger. To examine the impact of litigation, subjects and referents were divided into three groups, each related to the area where they lived. By adopting this approach, the sample size, and thus the power to detect effects, was considerably diminished and therefore does not represent a true assessment of the impact of litigation. For example, comparisons in zone A involved 9 nonclients versus 100 clients; corresponding figures for zone B were 18 versus 16 and for zone C

were 15 versus 11. The study reports mean values but not information on variability around the mean; in all other presentations, the standard deviations were shown. Thus, these two studies do not seem adequately suited to calculate trichloroethylene risk arising from chronic exposures.

A cross-sectional study of human environmental exposure was reported by Reif et al. (2003) based on residence in a community where the drinking water had been contaminated with trichloroethylene and related chemicals between 1981 and 1986 (Rocky Mountain Arsenal Superfund site). Tests of behavior, visual contrast sensitivity, and mood were carried out for estimated exposures of ≤5, >5-10, >10-15, and >15 ppb, with 5 ppb representing the maximum contaminant level for drinking water as defined by the EPA Office of Drinking Water. Testing occurred 6 years after peak concentrations of trichloroethylene. Subjects in the study (mean ages 48.6 to 55.8 years) resided in this area for a minimum of 2 years. In this analysis, trichloroethylene at >15 ppb affected visual function (contrast sensitivity) and increased scores for confusion, depression, and tension. For behavioral function, measured using the Neurobehavioral Core Test Battery, poorer performance on the digit symbol substitution test was reported. All these effects, however, were of marginal statistical significance. Further, these studies did not directly measure exposures but were based on estimates from geographic information systems. It is also not clear to what extent the participants were aware of exposures; cleanup began in 1986. In addition, there is no information on out-migration of the population (e.g., affected individuals who may have moved from the area). The role of duration of exposure was not evaluated. Moreover, the wells were contaminated with other organic solvents, although trichloroethylene was stated to be the primary contaminant and was present at high concentrations. Collectively or individually, these limitations could increase or decrease the sensitivity of this study to detect effects. For these reasons, including these data in the trichloroethylene risk assessment should be considered cautiously.

One interesting aspect of this study, however, if reliability of the trichloroethylene exposure assessments is assumed, is the strong interactions that emerged between trichloroethylene exposure and alcohol consumption. In the group exposed to trichloroethylene at >15 ppb, the impairments in the digit span test (considered a measure of memory) were highly significant among individuals reporting alcohol use of at least one drink per month compared with no alcohol use. In addition, these individuals showed longer simple reaction time values. Deficits in memory and response time are also characteristic of higher concentration, shorter-duration exposures. Thus, alcohol appeared to exaggerate some of the behavioral effects of trichloroethylene. These findings of alcohol-trichloroethylene interactions (see also Chapter 10), while intriguing, nevertheless require caution in interpretation based on the study limitations noted above.

MODE OF ACTION

As the current literature indicates, trichloroethylene has a wide array of effects on the nervous system that may involve different mechanisms of action. For example, changes in learning and memory might be related to the impact of trichloroethylene on long-term potentiation, considered to be a neurophysiological basis of learning. Current evidence also shows that trichloroethylene affects various neurotransmitter systems. Alterations in susceptibility to chemoconvulsants after trichloroethylene exposure implicates the involvement of GABA(A) receptors (Shih et al. 2001). Studies also show effects of trichloroethylene on

serotonin neurotransmitter systems (Gerlach et al. 1998; Lopreato et al. 2003). Dopaminergic consequences (e.g., Oshiro et al. 2004) likely contribute to motor deficits associated with trichloroethylene. Mechanisms of noncarcinogenic action for other target organs may likewise be operative in the brain, including oxidative stress. As a compound that can act on peroxisome proliferator-actived receptor α (PPARα), it is important to note the existence of such receptors in brain along with their functional roles as currently established (see Appendix E for some background information on PPARα agonism).

ISSUES

Trichloroethylene and Cognitive Function

The relationship between trichloroethylene exposure parameters and impairments of complex cognitive function remain unclear. White et al. (1997) described evidence in support of impaired cognitive function. In that study, neuropsychological testing was carried out in groups of exposed individuals from three different locations (Woburn, Massachusetts; Alpha, Ohio; and St. Paul-Minneapolis, Minnesota) and percentages of each group affected on different domains (relative to normative scores) were reported. Consistently affected across all three groups were attention and executive function and memory. Many details of this study were not reported (e.g., how subjects were recruited, awareness of exposure, litigation issues), nor were individual exposure measurements available for all subjects. Although White et al. (1997) stated that cognitive deficits were more pronounced the earlier in life the exposure occurred (developmental exposures are associated with more pronounced effects), they presented no data to support that assertion. The authors also noted that effects of these environmental (oral) exposures occurred at lower concentrations than anticipated from their experience with occupational cohorts; again, comparative data were not provided. Thus, while intriguing, it is difficult to determine the significance of the findings at the current time.

Studies reported in addition to those cited above (Oshiro et al. 2001, 2004) do little to clarify the question of the parameters of trichloroethylene exposure and impaired cognition. Isaacson et al. (1990) cited improvements rather than impairments in learning, here measured using a spatial learning paradigm in young male rats exposed to trichloroethylene in drinking water. Most improved were rats that had been exposed from day 21 to day 48 and again from day 63 to day 78 of age. Estimated intake of trichloroethylene in these studies averaged 5.5 mg/day for 28 days followed by 8.5 mg/day during the second exposure interval. Similarly, an unpublished study cited by Isaacson et al. (1990) apparently observed facilitation of learning in rats exposed to trichloroethylene during development.

Many noncognitive behavioral functions can indirectly influence measurement of learning (Cory-Slechta 1989). Alterations in motor function can alter the topography or effortfulness of responding. Sensory alterations can change the discriminability of environmental signals. Alterations in motivational state can influence either the salience or the potency of a reward. All these factors must also be controlled or explored in evaluating the outcome of learning paradigms. It is not possible to determine from the experimental description whether the facilitation noted by Isaacson et al. (1990) represents a true improvement in learning, or whether it is an indirect consequence of changes in other behavioral domains (e.g., faster swim time and thus shorter delay to reward).

An example described above comes from a report by Waseem et al. (2001) that examined neurobehavioral effects of trichloroethylene in rats after oral administration for 90 days of trichloroethylene at 350, 700, or 1,400 ppm or inhalation exposure at 376 ppm for 4 hours per day, 5 days per week for a total of 180 days. The report cited a lack of effect of exposure on acquisition of a conditioned shock-avoidance response. However, this study did not control for the potential of trichloroethylene to alter levels of shock sensitivity per se, which would thereby influence the rate of acquisition of this response.

In summary, it is not yet possible to ascertain the extent of trichloroethylene-induced impairment of complex functions such as learning, memory and attention, preferential vulnerability to trichloroethylene across these domains, the exposure parameters that might be associated with any adverse effects, the extent of their reversibility, and the impact of developmental period of exposure on such effects.

Sensitive Populations

Evidence to determine the extent to which trichloroethylene exposures during development or advanced age could enhance its adverse effects on the nervous system is limited. As noted above, White et al. (1997) reported more pronounced effects of environmental trichloroethylene exposures in younger humans, but they provided no data to support these statements. Experimental studies in which the effects of developmental exposures and adult trichloroethylene exposures are directly compared have not been reported. A study by Moser et al. (1999) of oral exposure to dichloroacetic acid, a metabolite that can be formed via mixed function oxidase metabolism of trichloroethylene, does include some comparisons of weanling versus adult rats. The results presented in the paper suggest comparable effects in the end points shown, although the authors noted that neuromuscular toxicity effects appeared to be somewhat greater in rats exposed as weanlings than in those exposed as adults. Generally speaking, there is insufficient evidence to ascertain whether there are developmental differences in sensitivity to trichloroethylene-induced neurotoxicity.

Aging does appear to enhance sensitivity to the adverse effects of trichloroethylene on the nervous system. A study by Arito et al. (1994) compared the responses of 2-, 13-, 20-, and 26-month-old rats to trichloroethylene at 300 ppm for 8 hours, followed by 1,000 ppm for 8 hours, after an intervening period of clean air for 7 days. In this study, the number of incidents of spontaneous bradyarrhythmia episodes during the 28-hour period after cessation of exposure to trichloroethylene at 300 or 1,000 ppm compared with those occurring during the corresponding period of exposure to clean air was significantly greater in 20- and 26-month-old rats than in the 2- or 13-month-old rats. Measurements of trichloroethylene in brain and blood also revealed a prolonged half-life and delayed clearance with advancing age, leading the authors to posit that pharmacokinetic differences during aging may contribute to this enhanced sensitivity. Certainly, aging needs to be considered in the uncertainties associated with the risk assessment calculations.

Interactions

The extent to which trichloroethylene neurotoxicity may be altered by coexposures with other environmental or dietary constitutents is not fully elaborated. One risk modifier for neurotoxicity described by Reif et al. (2003) is alcohol. As noted above, impairments in the digit span test in response to trichloroethylene were highly significant among individuals reporting alcohol consumption of at least one drink per month, whereas no effects were observed in individuals reporting no alcohol consumption. In addition, individuals who consumed alcohol also showed longer simple reaction time values in response to trichloroethylene exposure. Thus, alcohol was noted to potentiate the effects of trichloroethylene on a measure of attention and memory. As noted previously, however, limitations of this study necessitate caution in interpreting the validity of these findings.

Significance of Brain Concentrations of Trichloroethylene

Boyes et al. (2000, 2003) explored the relationship between exposure concentration and duration under conditions of acute exposure in predicting risk of trichloroethylene neurotoxicity. The first of these studies (Boyes et al. 2000), examining hearing loss, signal detection behavior, and visual function, demonstrated that Haber's law would overestimate when extrapolating from shorter to longer trichloroethylene exposure durations and would underestimate when extrapolating from longer to shorter exposures. This study also showed that, instead, the estimated peak blood concentration of trichloroethylene at the time of testing accurately predicted the magnitude of effect on visual function and on signal detection (these neurotoxic effects reflect momentary tissue concentrations of trichloroethylene). A second study (Boyes et al. 2003) used visual evoked potentials as an outcome measure and demonstrated that the exposure metric of area under the curve was not an accurate predictor of effect; instead, brain concentration of trichloroethylene at the time of visual evoked potential testing predicted the effects of trichloroethylene across exposure concentration-duration parameters. Still questionable, however, is the extent to which this relationship would generalize to longer-term exposures.

Neurodegenerative Disease

Some reports have suggested a link between trichloroethylene exposure and Parkinson's disease. Among these are two case reports. Guehl et al. (1999) described a case of Parkinson's disease in a 47-year-old woman who had 7 years of exposure to trichloroethylene. This case is notable given the young age relative to typical onset and the fact that the subject was a woman, because time to onset is longer and Parkinson's disease incidence is lower in women than in men. Unknown, however, is the specificity of the exposure and the genetic background of the subjects. In another report, Kochen et al. (2003) cited the onset of Parkinson's disease in three individuals chronically exposed to trichloroethylene during the postexposure period. Following the case report observation, Guehl et al. (1999) also described the loss of dopamine neurons in substantia nigra pars compacta (the hallmark of Parkinson's disease) after intraperitoneal

injections of trichloroethylene at 400 mg/kg per day, 5 days a week for 4 weeks, to mice. No follow up studies to this report have been described.

Although it is not clear whether assessment of the incidence of Parkinson's disease has been examined in trichloroethylene-exposed populations, a biological basis for its potential contribution to this disease has been suggested by Riederer et al. (2002) to be based on the formation of TaClo (1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline), a potent dopaminergic neurotoxin that can be formed endogenously after exposure to the sedative chloral hydrate or after exposure to trichloroethylene. As the authors note, trichloroethylene has an estimated half-life in humans in venous blood of 21.7 hours, sufficient for the appropriate in vivo condensation reactions that would be involved in TaClo formation. Indeed, significant amounts of TaClo (approximately 200 ng per 10 mL samples) were detected in both serum and clot in Parkinson's disease patients that had been treated for several days with 500 mg of chloral hydrate (a metabolite of trichloroethylene), with blood sampled on the final day of treatment (Bringmann et al. 1999).

Additionally, there are significant structural similarities between TaClo and MPP^+ (1-methyl-4-phenylpyridinium ion), the most widely used experimental model for Parkinson's disease. As noted in the review by Riederer et al. (2002), like MPP^+, TaClo specifically inhibits the electron transfer from complex I to ubiquinone in the mitochondrial respiratory chain in both rat brain homogenate and rat liver submitochondrial particle preparations. TaClo was shown to reduce dopamine uptake and the number and size of tyrosine-hydroxylase-positive cells in C56/BL6 mouse primary cell cultures. Injection of TaClo directly into the rat substantia nigra pars compacta decreases both neuronal density and number of neurons in this region. This treatment also resulted in a progressive decline in concentrations of the dopamine metabolite 3,4-dihydroxyphenylacetic acid over 6 weeks after a single injection, consistent with some other models of the Parkinson's disease phenotype. While an intriguing series of studies, it is unknown whether TaClo can be formed following trichloroethylene exposure per se.

FINDINGS AND RECOMMENDATIONS

With respect to the EPA (2001b) draft risk assessment, several neurotoxicity studies contributed to the derivation of an inhalation RfC. In general, these studies report effects in humans and in experimental models (rat) at very similar concentrations. In addition, common effects are seen across these studies, and the nature of the effects described are comparable to or consistent with those reported in response to acute exposures to higher concentrations. Those studies utilized to derive the RfC include reports in humans of changes in trigeminal nerve function (measured using the massiter reflex latency) and motor incoordination at human equivalent LOAEL concentrations of 7-16 ppm (Ruijten et al. 1991; Rasmussen et al. 1993) and symptoms including nausea, drowsiness, and fatigue (Okawa and Bodner 1973; Vandervort and Polakoff 1973). Studies in rats showed changes in heart rate and wakefulness at a human pharmacokinetic adjusted LOAEL of 9 ppm (Arito et al. 1994). This appears to be a valid and standard approach taken to evaluate risk.

Furthermore, as is clear from the discussions above, new information on trichloroethylene published since the EPA (2001b) review is limited and thus may offer little in the way of amendment to the current RfC: